the Energy to Lead

QUARTERLY REPORT

GTI PROJECT NUMBER 20916

Modeling of Microbial Induced Corrosion on Metallic Pipelines Resulting from Biomethane and the Integrity Impact of Biomethane on Non-Metallic Pipelines

DOT Prj# 293

Contract Number: DTPH56-09-T-000002

Reporting Period:
January 1, 2010 – March 31, 2010

Prepared For:
James Merritt
Department of Transportation
Pipeline & Hazardous Materials Safety Administration
Office of Pipeline Safety
Email james.merritt@dot.gov
Telephone: (303) 683-3117

Prepared By:

GTI Project Team:
Karen Crippen, Daniel Ersoy, Monica Ferrer, Zhongquan Zhou, Xiangyang Zhu

Kristine Cruz, *Team Project Manager*
Kristine.cruz@gastechnology.org
847-768-0910

Gas Technology Institute
1700 S. Mount Prospect Rd.
Des Plaines, Illinois 60018
www.gastechnology.org

Legal Notice

This information was prepared by Gas Technology Institute ("GTI") for DOT/PHMSA (Contract Number: DTPH56-09-T-000002.

Neither GTI, the members of GTI, the Sponsor(s), nor any person acting on behalf of any of them:

a. Makes any warranty or representation, express or implied with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately-owned rights. Inasmuch as this project is experimental in nature, the technical information, results, or conclusions cannot be predicted. Conclusions and analysis of results by GTI represent GTI's opinion based on inferences from measurements and empirical relationships, which inferences and assumptions are not infallible, and with respect to which competent specialists may differ.

b. Assumes any liability with respect to the use of, or for any and all damages resulting from the use of, any information, apparatus, method, or process disclosed in this report; any other use of, or reliance on, this report by any third party is at the third party's sole risk.

c. The results within this report relate only to the items tested.

Table of Contents

Table of Tables

Page

Table of Figures

Executive Summary

The objective of this project is to understand key elements related to promoting the successful delivery of biomethane into natural gas pipeline networks. This project focuses on two key areas of concern: [1] the effect of microbial induced corrosion on metallic pipes and [2] the impacts of biogas/biomethane on a non-metallic gathering network from sustained biogas feedstock exposure. This report summarizes the work that has been conducted through the first quarter of 2010. Results from Tasks 1, 2, 3 and 5 are discussed in detail within this report.

Task 1. Literature Review

Task 1 includes a comprehensive review of biological and chemical information as it relates to the two focus areas of this project: [1] the effect of microbial induced corrosion on metallic pipes and [2] the impacts of biogas/biomethane on a non-metallic gathering network from sustained biogas feedstock exposure.

A comprehensive literature review of publications, standard documents, research reports, and publications in scientific journals was conducted on the topic of internal MIC. The literature review provides an overview of corrosion and MIC related microorganisms. The literature review also provides specific information about MIC detection and limitation, MIC mitigation and prevention, and their relationship to overall pipeline corrosion, as well as those major factors or mechanisms which control the internal MIC process on metallic pipelines. The literature review in addition to the data obtained from Task 2 will identify a set of major parameters for the construction of a preliminary MIC model that will be developed in Task 4.

Task 1 also includes the collection of chemical data from biogas and biomethane derived from dairy manure, landfills, and wastewater treatment plants (WWTP). Data was obtained from previous GTI projects as well as samples sent to GTI's analytical laboratory from industrial customers. In addition, an extensive literature review was performed and several datasets from biogas facilities were obtained. This data will be compared with comprehensive natural gas data sets acquired over the past two decades.

The data collected from previous GTI projects, GTI analytical laboratory results from industrial customers, and literature search results have been complied into table format and is included in the Appendices. Appendices A- C includes *First Tier* compounds from biogas, biomethane and natural gas, respectively. Appendices D-F includes *Second Tier* compounds from biogas, biomethane and natural gas, respectively. Table 6 within the report summarizes the number of samples collected for review based on the type of gas and source of information.

Task 2. Microbial/Chemical Profile of a Metallic Biogas Pipeline

The results from several biological analyses are presented in this report: [1] the number of total (live and dead) heterotrophic bacteria and various corrosion causing bacteria (APB, IOB, and SRB), [2] the number and identity of live bacteria, and [3] the number and identity of bacterial spores. The number of total bacteria and total corrosion-causing bacteria including both dead and live bacteria was determined using a genetic method (qPCR) by targeting specific genes present in the target microorganisms. The DNA from the above sources was used to determine the identities of heterotrophic bacteria, bacterial spores, and corrosion-related APB and IOB.

These results will be used to formulate a synthetic condensate and bacteria consortium for Task 3 experiments to determine the corrosion effect of microbes on metal pipelines and to collect data for the MIC modeling that will be conducted in Task 4.

Task 3. Lab Evaluation of Microbial Corrosion under Simulated Field Conditions

The accurate diagnosis of MIC requires a combination of microbiological, chemical, and metallurgical analyses. The microbiological indicators include detection and quantification of various microorganisms on metal-liquid interface, especially corrosive bacteria in biofilms formed on metal surfaces. In this task, major microbial and chemical parameters have been identified and will be included in the Task 4 modeling experiments.

Major Microbial Parameters

The major bacterial populations in raw biogas and condensate samples collected from gathering lines have been determined in Task 2, and the results have been used to formulate a major corrosion-related bacteria consortium to evaluate the microbial corrosion of metallic pipelines. In addition, chemical compositions and properties of typical condensate in a raw biogas gathering line will be thoroughly analyzed in Task 2. Therefore, the microbial corrosion evaluation will be performed using a synthetic condensate to mimic the field conditions typically found in a raw biogas gathering line. The bacteria consortium for modeling of MIC in a raw biogas gathering line will consist of *B. licheniformis* ATCC 14580, *Paenibacillus barengoltzii*, *P. glucanolyticus*, and *C. acetobutylicum* ATCC 824.

Major Chemical Parameters

Water is required for microbial metabolism and growth and corrosion processes. Water quality parameters that are considered important to understanding internal corrosion and MIC for a particular industrial system include: temperature, pH, alkalinity, sulfide, nitrite, dissolved gases (CO_2, H_2S, O_2, NH_3, etc.), total dissolved solid (TDS), chemical oxygen demand (COD), microorganisms (bacteria, algae, and fungi), etc.

Pope and Pope (Pope and Pope 1998) listed a series of chemical and metallurgical indicators for diagnosis of MIC in natural gas pipelines. Chemical MIC indicators include: sulfide, sulfate, chlorides, short-chain volatile fatty acids, pH, ferrous iron, ferric iron, hardness, and carbonate. Metallurgical MIC indicators include: discrete deposits, deposit color, under deposit pit, shiny pit, and larger pits composed of smaller pits.

Task 5. Develop Compilation of Nonmetallic Materials

A review of the nonmetallic materials that are currently used for building natural gas distribution systems were performed in this quarter. The overview of plastic pipes, together with the physical and mechanical properties and pipe joining methods are summarized in this report. A compilation of the elastomeric materials that are used as gasket, seal, etc for natural gas distribution systems are generated and the general description of each material including the physical, mechanical properties and chemical and environmental resistance are included. More detail analysis of each material will be performed in the next quarter to obtain a complete compilation of the material properties and their performance under the potential environment which may be encountered for biogas/biomethane applications.

List Activities/Deliverables Completed During Reporting Period

	SCH Date	CMPL Date
Task #1: Literature Review	6/30/2010	In Progress
Task #2: Microbial/Chemical Profile of a Metallic Biogas Pipeline	6/30/2010	In Progress
Task #5: Develop Compilation of Non-metallic Materials	6/30/2010	In Progress

Technical Status

1.1.1 *Task 1 - Literature Review of Internal Microbial Corrosion*

Microbiologically influenced corrosion (MIC) is a complex and aggressive mode of corrosion (Zhu; King, Miller et al. 1973; King and Wakerley 1973; Hamilton 1985; Pope, Zintel et al. 1989; Angostini and Young 1990; Dzierzewicz 1992; Emde 1992; Strickland 1996; Dzierzewicz, Cwalina et al. 1997; Farthing 1997; Jack, Wilmott et al. 1998; Pope and Pope 1998; Angell 1999; Li, Kim et al. 2001; Horn and Jones 2002; Kane and Campbell 2004; Zhu, Modi et al. 2006; Zhu 2007). A comprehensive literature review of publications, standard documents, research reports, and publications in scientific journals was conducted on the topic of internal MIC over a nine-month period. The literature review is focused on information about MIC detection and limitation, MIC mitigation and prevention, and their relationship to overall pipeline corrosion, as well as those major factors or mechanisms which control the internal MIC process on metallic pipelines. The second focus of the literature review is to incorporate the data from Task 2 (conditions in raw biogas gathering line) and discuss its implications for potential microbial corrosion. The literature review will identify a set of major parameters for the construction of a preliminary MIC model in Task 4.

Background

Corrosion is mainly the consequence of electrochemical reactions on the surface of a metal. Its kinetics are determined by the physical/chemical environment at the metal surface, such as concentration of oxygen, salts, pH, reduction-oxidation (redox) potential, and conductivity (Figure 1). Microbiologically influenced corrosion (MIC) is corrosion influenced by the presence or activities of microorganisms including bacteria and fungi (de Franca and Lutterbach 1996; Batista 2000; Pope and Pope 2001; NACE 2006). Microorganisms growing at the metal surface form a biofilm and the release of chemicals or the deposition of electrochemically active minerals from biofilms alters the rates and types of electrochemical reactions at the biofilm-metal surface interface and produces a broad range of

Figure 1. Internal Microbial Corrosion.

outcomes such as pitting, crevice corrosion, under-deposit corrosion, selective dealloying, enhanced erosion, and galvanic corrosion (Kasahara and Kajiyama 1986; Pope, Zintel et al. 1988; Little, Wagner et al. 1998; Pope 2001; Shi, Avci et al. 2002; NACE 2006; Waters, El-Naggar et al. 2009) (Figure 2). The accurate diagnosis of MIC requires a combination of microbiological, surface analytical and electrochemical techniques.

Despite the tremendous advances made in recent years to improve knowledge of the mechanisms of microbial corrosion, and development of better monitoring techniques, biocides, and other control measures, it is still not known with certainty how many species of microorganisms contribute to corrosion, how to reliably detect their presence prior to corrosion events, or how to rapidly assess the efficacy of mitigation procedures (Pope 1989; Emde 1992; Graves 1996; Strickland 1996; Farthing 1997; Pope 1998; Angell 1999; Batista 2000; Kholodenko 2000).

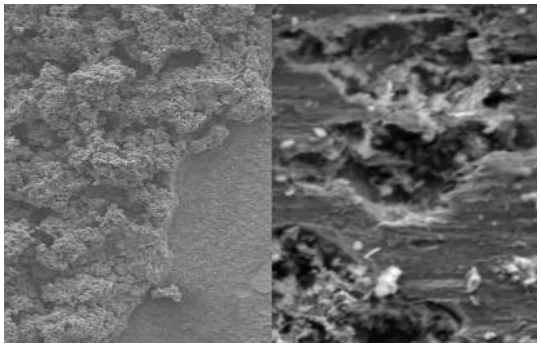

Figure 2. SEM Micrograph of Biofilm and Microbial Corrosion.

MIC can occur in unexpected places. It tends to occur repeatedly at certain locations (Table 1) (Scott 2004). In general, MIC "problem areas" for many industries occur more often in welds and heat-affected zones, separators, drips, under film deposits, after hydrotesting, and when cooling systems are not passivated after "turnarounds" are complete.

MIC-Related Microorganisms and MIC Mechanisms

Many bacteria occurring naturally in waters and soils are considered corrosion-causing bacteria, including but not limited to, sulfate-reducing bacteria (SRB), acid-producing bacteria (APB), metal-oxidizing bacteria (MOB), metal-reducing bacteria (MRB), sulfur/sulfide oxidizing bacteria, nitrate-reducing bacteria, and slime-forming bacteria. Each of these physiological groups of microorganisms may contain hundreds or thousands of individual species. Each group of bacteria or an individual species of bacteria alone can cause metal corrosion; however in a natural environment, it is always microbial communities containing many different types of microbes that cause the MIC, and the resulting corrosion is always far more severe compared to the data generated under single strain laboratory conditions (Pope and Pope 1998). However, the mere presence of given classes of organisms associated with MIC (e.g., SRB) does not necessarily indicate that MIC is occurring. Nor does the showing that a given type of microorganisms is present establish a cause-and-effect relationship between the bacteria and metal dissolution (Jack, Rogoz et al. 1994; Horn and Jones 2002).

Many MIC mechanisms have been proposed since von Wolzgen Kuhr and Van Der Vlugt in 1934 (von Wolzogen Kuhr and van der Vlugt 1934); most of them are focused on SRB corrosion (Zhu; King, Miller et al. 1973; Hardy and Brown 1984; McNeil and Little 1990; Jack and Wilmott 1995; Jack, Wilmott et al. 1998; Tributsch, Rojas-Chapana et al. 1998; Li, Kim et al. 2001). A general mechanistic MIC model proposed by Pope includes three phases (Pope and III 1995; Pope 2001) (Figure 3) . In Phase I, microbes attach to metal surface and start forming a

biofilm. The attachment colonization of microbes in this phase is affected by many conditions such as preexisting corrosion on the metal surface, metal surface condition (roughness, welds, inclusions, etc.), and local chemical-electrochemical environments. The further development of biofilm on metal surface in Phase II creates an occluded area (inside and under the biofilms) that is relatively anodic to the surrounding area. In this phase, the occluded area becomes more acidic, attracting chloride and other anions and starts forming deposits on the metal surface (nodules or tubercles). Phase III involves the formation of a mature nodule over a well-defined pit. The low pH (<4.0) in the active pit region shifts the corrosion process to chemically-driven underdeposit acid attack. In this phase, the corrosion process would continue even in the absence of microbes (Pope and III 1995).

Table 1. Where MIC is most likely to occur (Scott 2004).

Industry/Application	Potential Problem Sites for MIC	Organisms Responsible
Pipelines-oil, gas, water, wastewater	Internal corrosion primarily at the bottom position Dead ends and stagnant areas Low points in long-distance pipes	Aerobic and anaerobic acid producers, SRB, manganese and iron-oxidizing bacteria, sulfur oxidizing bacteria
Chemical process industry	Heat exchangers, condensers, and storage tanks-especially at the bottom where there is sludge build-up Water distribution systems	Aerobic and anaerobic acid producers, SRB, manganese, and iron-oxidizing bacteria In oil storage tanks also methanogens, oil-hydrolyzing bacteria
Cooling water systems	Cooling towers Heat exchangers-in tubes and welded areas-on shell where water is on shell side	Algae, fungi, and other microorganisms in cooling towers Slime-forming bacteria, aerobic and anaerobic bacteria, metal-oxidizing bacteria, and other microorganisms and invertebrates
Fire protection systems	Dead ends and stagnant areas	Anaerobic bacteria, including SRB
Docks, piers, oil platforms, and other aquatic structures	Just below the low-tide line Splash zone	SRB below barnacles, mussels, and other areas sequestered from oxygen
Pulp and paper	Rotating cylinder machines Whitewater clarifiers	Slime-forming bacteria and fungi on paper-making machines Iron-oxidizing bacteria SRB in waste
Power generation plants	Heat exchangers and condensers Firewater distribution systems Intakes	As above for heat exchangers and fire protection systems Under mussels and other fouling organisms on intakes
Desalonation	Biofilm development on reverse osmosis membranes	Slime-forming bacteria

However, in a complex environment, a consortium of different types of microorganisms often work synergistically, resulting in far more severe corrosion compared to the data generated under single strain laboratory conditions (Pope and Pope 1998). For instance, APB produce low molecular weight organic acids (short chain fatty acids such as acetic, butyric, formic, lactic, succinic, and propionic acids) and inorganic acids (e.g., HCl, H_2CO_3, and H_2SO_4). While both types of acids can cause metal corrosion by either direct reaction with metal or disrupting the protective surface oxides films and calcium scales (Gibson and Wang 1994; Zellner, Stackebrandt et al. 1996; Kanauchi, Fujiyama et al. 1999; Broda, Saul et al. 2000; Vetting, D'Argenio et al. 2000; Horn and Jones 2002; Zhu, Lubeck et al. 2003; NACE 2006), the organic

acids provide the environment and nutrients for the growth of other bacteria such as SRB (Little and Wagner 2001) (Figure 4). In addition, biogenic acids increase the concentration of protons (H^+), which can then become reduced at the cathode, generating hydrogen, an electron source for SRB and other hydrogen-consuming organisms (Horn and Jones 2002). Activities of aerobic microbes deplete oxygen in the biofilm, create an environment for growth of anaerobic bacteria, and form an oxygen gradient within the biofilm. This causes a potential change beneath the film, resulting in the development of an anodic region surrounded by a large cathodic area and galvanic corrosion. In addition, if the protective oxide film is breached beneath a biofilm, then the metal cannot be reoxidize or self-heal. Oxygen gradients and breached oxide film result in metal pitting beneath biofilms. Therefore MIC is the consequence of collective effects of microbial consortia on metal surfaces.

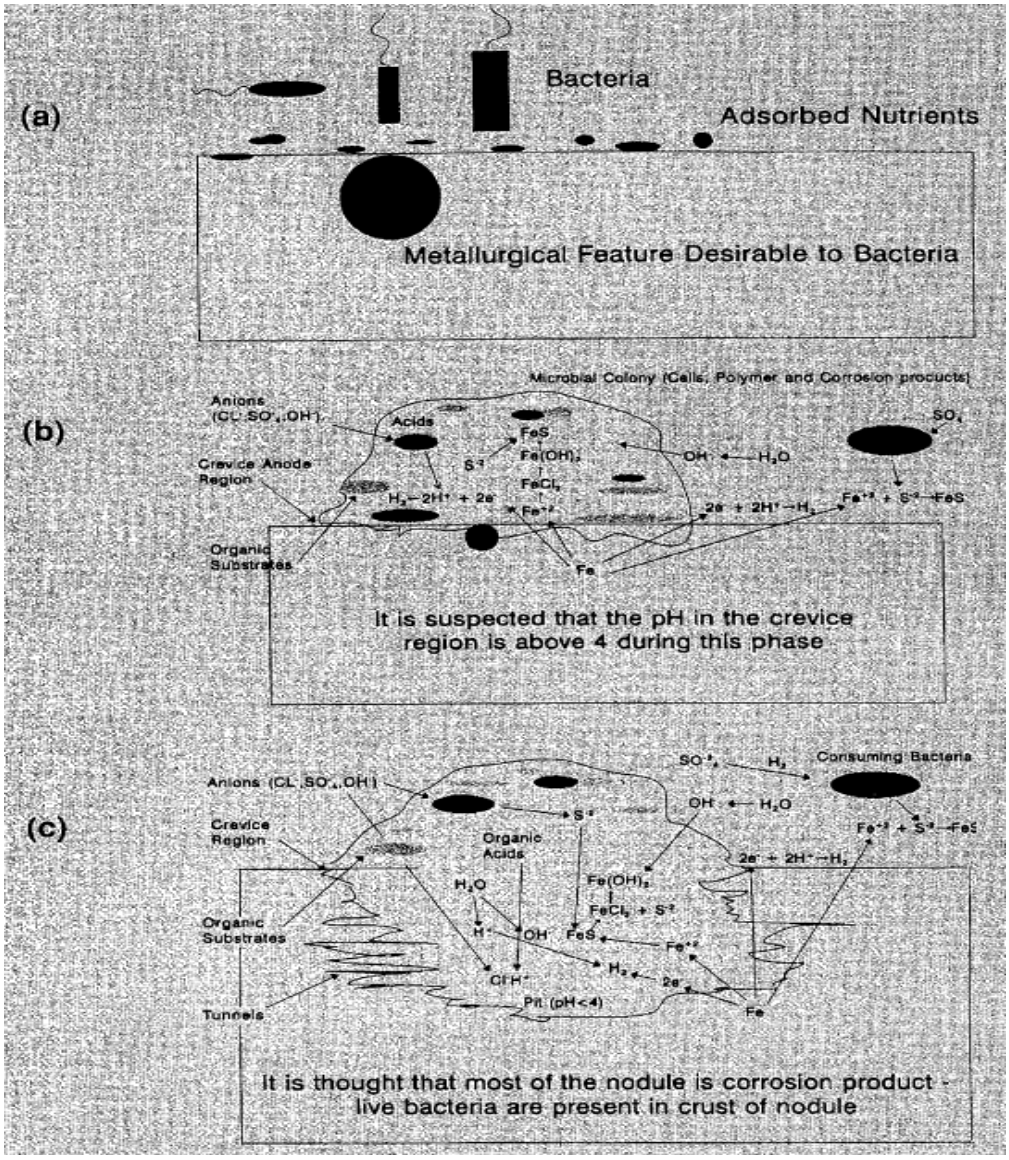

Figure 3. MIC Development Model (Pope and III 1995). (a) Recognition of Desirable Sites. (b) Colony Formation and Crevice Corrosion Begins and Anode is Fixed. (c) Nodule is Formed over "Mature" Pit.

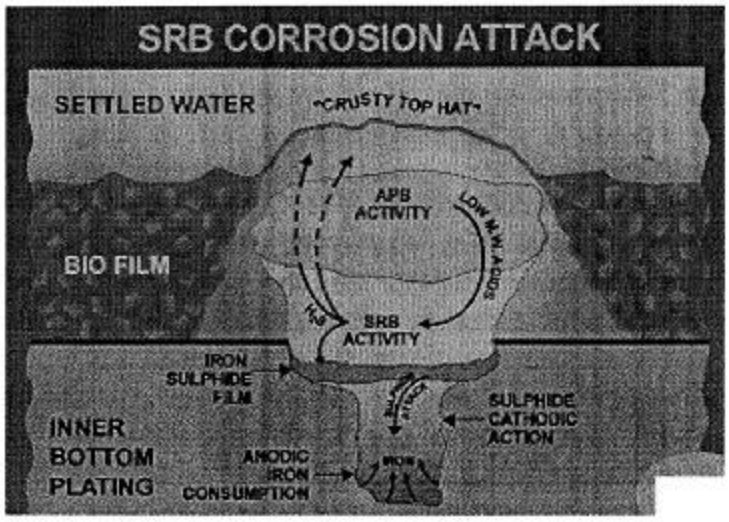

Figure 4. Interaction of SRB and APB on Metal Corrosion (Towers 2000).

1.1.1.1.1 SRB-induced corrosion

Sulfate-reducing bacteria (SRB) constitute a physiologically diverse group of obligate anaerobic, heterotrophic, and mixotrophic bacteria that are responsible for dissimilatory sulfate reduction. They are present in a variety of environments, including oil- and gas-bearing formations, seawater, freshwater, soils, and domestic, industrial, and mining wastewaters (Hines, Visscher et al. 2002). Though SRB are anaerobic bacteria, SRB can survive and quickly recover from brief oxygen exposure (Hardy and Brown 1984; Towers 2000; Zhu, Modi et al. 2006). SRB use hydrogen, organic acids (lactic, acetic, propionic, succinic, pyruvic, etc.), and variety of other low molecular weight organic compounds (ethanol, aliphatic acids, sugars, amino acids, indole, nicotinic acid, etc.) as electron donors and also as carbon and energy sources. Sulfate can be used as an electron acceptor for anaerobic respiration (Bak and N. 1991; Caumette 1993; Mudryk, Podgórska et al. 2000). Previous microbiological studies have suggested that SRB play a key role in microbial corrosion (Graves 1996; Pope and Pope 1998) and other problems of great economic impact in oil and gas industries (Graves 1996). For instance, oil reservoir souring is a well known phenomenon after seawater injection into reservoirs for oil extraction, i.e., the reservoir formation water provides volatile fatty acids (VFAs) as electron donors and the seawater provides the sulfate (~2,700 mg/L) as electron acceptor for SRB's anaerobic respiration.

It has been reported by many researchers that the corrosion rates caused by SRB under laboratory conditions are much lower than the rates under field conditions (King, Miller et al. 1973; King and Wakerley 1973; Hamilton 1985; Jack, Wilmott et al. 1998; Tributsch, Rojas-Chapana et al. 1998; Li, Kim et al. 2001; Kane and Campbell 2004; Zhu, Modi et al. 2006; Le Borgne, Romero et al. 2007), and the rates under laboratory conditions usually cannot be maintained at high level for long periods of time. The existence and activity of SRB causes the average corrosion rate of steel exposed to anaerobic soil to be more than 20 times higher than that of the control case, the maximum corrosion rate of steel and iron being reported by SRB to be 7.4 mm/y (Kasahara and Kajiyama 1986; Jack 2001; Li, Kim et al. 2001). Pitting corrosion is characteristic of the action of SRBs on steel, with pits being open and filled with soft black

corrosion products in the form of iron sulfides (Hamilton 1985). When the corrosion products are removed, the metal underneath is bright but rapidly rusts on exposure to air.

Various mechanisms have been proposed to explain the accelerated corrosion rate observed in the presence of SRB. The most classic among them is cathodic depolarization, proposed by Von Wolzogen Kühr and Van Der Vlugt in 1934 (von Wolzogen Kuhr and van der Vlugt 1934). They proposed that cathodic depolarization is achieved by the metabolic oxidation of hydrogen by SRBs.

$4Fe \rightarrow 4Fe^{+2} + 8e^{-}$	(anodic reaction)	(1)
$8H_2O \rightarrow 8H^{+} + 8OH^{-}$	(water dissociation)	(2)
$8H^{+} + 8e^{-} \rightarrow 8H^{0}$	(cathodic reaction)	(3)
$SO_4^{-2} + 8H^{0}$ MIC $\rightarrow S^{-2} + 4H_2O$	(cathodic depolarization)	(4)
$Fe^{2+} + S^{-2} \rightarrow FeS$	(corrosion products)	(5)
$3Fe^{+2} + 6OH^{-} \rightarrow 3Fe(OH)_2$	(corrosion products)	(6)
$4Fe + SO_4^{-2} + 4H_2O \rightarrow 3Fe(OH)_2 + FeS + 2OH^{-}$	(overall reaction)	(7)

The cathodic depolarization theory posits that SRB at the cathode remove the H^0 from a polarized metal surface (through hydrogenase) for anaerobic respiration (to produce energy by reducing sulfate to sulfide), resulting in increased corrosion rate. However, many later researchers found evidence that conflicts with cathodic depolarization hypothesis (Hamilton 1985). It has been reported that the reactions occurring at the anode are at least as important as the cathode's and could be predominant in the case of SRB corrosion (Campaignolle, Luo et al. 1993).

The most severe damage resulting from the corrosion of steel by SRB is most often localized, taking the form of pits, crates or similar clearly delimited areas of corrosion. Pitting corrosion is a process of the nucleation and growth type, and the mechanism of pitting corrosion is generally an autocatalytic stabilization of a galvanic cell between a small corroding area (the anode) and its non-corroding surroundings (the cathode). Thus, the more modern theory of SRB-induced corrosion involves the formation of ferrous sulfide film on metal surface and the formation of galvanic cell between ferrous sulfide film and steel base.

The galvanic corrosion theory states that under anaerobic conditions, SRB uses various electron donors (mainly small molecule organic acids) to reduce inorganic sulfate to sulfide. As a result, hydrogen sulfide accumulates in the biofilm near the metal surface and iron sulfide quickly forms on and covers the carbon steel surface. The iron sulfide film (cathode) and bare steel base (anode) forms a galvanic cell (Daumas, Magot et al. 1993). At the early stage, the film (mainly mackinawite, $FeS_{(1-x)}$, 35% S, dense and protective) is patchy and irregular, and therefore SRB-induced corrosion rates are high due to the galvanic couple between patchy iron sulfide (cathode) and steel base (anode). However, after a uniform mackinawite film is formed, it protects metal from further corrosion, resulting in reduced SRB corrosion (Kasahara and Kajiyama 1986). When mackinawite takes up more sulfide and gradually converts to greigite (Fe_3S_4) and pyrite (FeS_2, 52.5% S), the change in film density breaks the iron sulfide film and the resulting ruptured film exposes the bare metal, forms a galvanic corrosion cell again between the steel substrate and an unbroken sulfide film attached to the steel surface, and causes elevated

corrosion rate (Hemmingsen, Vangdal et al. 1992; Jack, Wilmott et al. 1998). Pyrite is 12 times more corrosive than mackinawite due to higher potential difference to the iron anode (482 mV vs 610 mV). However, the incubation time for breakdown of mackinawite film, dependant on various factors such as redox potential, solution chemistry, physical properties of films, is not predictable, and may take 2-3 months (King, Miller et al. 1973; Li, Kim et al. 2001). A high concentration of ferrous iron in the medium may accelerate the breakdown of dense biogenic FeS film on the metal surface, and accelerate the corrosion rate (Zhu; King, Miller et al. 1973). High amounts of soluble iron also prevent formation of protective sulfide layers on ferrous metals (King and Wakerley 1973). Once mackinawite film is ruptured, the corrosion is independent of SRB number and growth rate.

The galvanic corrosion cell is normally short lived because the iron sulfide matrix becomes saturated with electrons derived from the corrosion process. However, anaerobic SRB remove electrons directly from FeS_x matrix (cathode), sustaining a flow of electrons through the galvanic couple from the corroding steel (Jack, Wilmott et al. 1998). The microbes use these electrons to reduce sulfate to sulfide, which combines with ferrous ions (Fe^{2+}) derived from corrosion of the steel to precipitate more FeS_x, thus further increasing corrosive action. Other researchers found that the activity of the SRB on the anode (electrochemical or metabolic) might be more important than their activity on the cathode in terms of stabilizing the coupling current between the anode and the cathode, and proposed a theory that the SRB acidify the anode by precipitating ferrous ions into ferrous sulfide and stabilize the pH of the cathode, thus inducing a sustained galvanic coupling (Campaignolle, Luo et al. 1993; Daumas, Magot et al. 1993; Campaignolle and Crolet 1997; Tanji, Itoh et al. 2002). The galvanic couple accounts for ~ 10% of the observed damage. Extension of the life of the corrosion cell through electron transfer to active bacteria is responsible for most of the metal loss (Jack, Wilmott et al. 1998). Another classic hypothesis regarding the sustaining galvanic corrosion cell was proposed by King and Miller (King and Miller 1971; King, Miller et al. 1973; Kasahara and Kajiyama 1986). They attribute the sustaining life of galvanic cell to the adsorption of atomic hydrogen by the ferrous sulfide corrosion product. Ferrous sulfide is not, however, a permanent cathode (King, Miller et al. 1973) and its regeneration and the maintenance of a high sustained corrosion rate is dependent on the removal of this hydrogen by the action of bacterial hydrogenase.

Other alternative hypotheses also exist, and may contribute to the explanation of SRB-induced corrosion. For instance, some SRB secrete exopolysaccharides (EPS), which facilitates irreversible cell attachment, leading to colonization on the steel surface. EPS can bind metal ions, causing metal ion concentration cells (Angeles-Ch, Mora-Mendoza et al. 2002). Hydrogen sulfide acidifies a corrosive medium and catalyzes penetration of hydrogen into steels, a process known as H_2S-induced cracking or sulfide stress cracking (Gonzalez, Ramirez et al. 1997; Little, Ray et al. 2000). Periodic oxygen incursions and sulfur/sulfide oxidizing bacteria can oxidize FeS_x to more corrosive sulfides such as pyrite (higher sulfur content) and production of elemental sulfur ($2S^{2-} + O_2 + 4H^+ \rightarrow 2S^{(0)} + 2H_2O$). Both products will increase corrosion significantly (Zhu; Hardy and Brown 1984; Zhu, Modi et al. 2006). Elemental sulfur sustains the galvanic couple between iron and the corrosion product FeS_x by accepting electrons from the FeS_x. High local acidity generated on particles of solid sulfur reacting with water could also be responsible for high corrosion rates of iron and steel.

1.1.1.1.2 APB-induced corrosion

Acid-producing bacteria (APB) are present in a variety of environments, including oi1- and gas-bearing formations, soils, and domestic, industrial and mining wastewaters. Acid-producing bacteria produce organic acids (e.g., acetic, butyric, formtic, lactic, succinic, and propionic acids) and inorganic acids (e.g., HCl, H_2CO_3, H_2SO_4), causing metal corrosion by either direct reaction with metal or disrupting the protective surface oxides films and calcium scales (Gibson and Wang 1994; Zellner, Stackebrandt et al. 1996; Kanauchi, Fujiyama et al. 1999; Broda, Saul et al. 2000; Vetting, D'Argenio et al. 2000; Horn and Jones 2002; Zhu, Lubeck et al. 2003; NACE 2006). In addition, biogenic acids increase the concentration of protons (H^+), which can then become reduced at the cathode, generating hydrogen, an electron source for SRB and other hydrogen-consuming organisms (Daumas, Magot et al. 1993; Horn and Jones 2002). Short chain organic acids provide the nutrients for other bacteria growth such as SRB and can lead to general attack, pitting attack, and stress corrosion cracking (Little and Wagner 2001). Acetic acid-producing bacteria and butyric acid-producing bacteria have been found to be present in environmental samples and in particular, samples from gas and oil production operations (Pope, Zintel et al. 1989; Zhu 2007; Zhu 2008). Consumption of hydrogen by SRB through formation of H_2S allows the APB to continue acid production. Some fungi also produce organic acids and other byproducts which support the growth of various other bacteria such as SRB (NACE 2006).

1.1.1.1.3 MOB- induced corrosion

Metal-oxidizing bacteria (MOB), mainly iron-oxidizing bacteria and manganese-oxidizing bacteria, are generally filamentous, are typically found in fresh and marine water, and are frequently surrounded by a sheath usually encrusted with iron, manganese, or both. Iron-oxidizing bacteria such as *Gallionella, Sphaerotilus, Leptothrix, Siderocapsa, Thiobacillus, Crenothrix*, and *Clonothrix* oxidize the soluble ferrous (Fe^{2+}) and produce orange-red tubercles of iron oxides and hydroxides by oxidizing ferrous ions (electron donors) from the bulk medium or the substratum (Hanert 1981; Emerson and Ghiorse 1992). They are commonly associated with tubercle formation and corrosion of water distribution pipelines. The small area under the deposit, deprived of oxygen, forms a galvanic cell with surrounding metal with large cathode to anode ratio, resulting in under-deposit corrosion, pitting, and crevice corrosion (Dickinson and Lewandowski 1996; NACE 2006), sometimes with assistance from sulfate-reducing bacteria (Hamilton 1985). *Gallionella* spp. contributes to the generation of conditions favorable to colonization by SRB (de Franca and Lutterbach 1996). Manganese-oxidizing bacteria oxidize the soluble manganese (Mn^{2+}) to insoluble manganese oxide (Mn_2O_3, MnOOH, Mn_3O_4, and MnO_2). The oxides are formed extracellularly and encrust the polymeric material (bacterial capsules) that surrounds individual cells or cell aggregates. *Leptothrix* and *Siderocapsa* are particularly associated with formation of highly enriched manganese oxide deposits. Manganese oxide can elevate corrosion current, and can also serve as a cathode to support corrosion at an oxygen depleted anode (metal surface) within the deposit, resulting in similar under-deposit corrosion, pitting, and crevice corrosion (Dickinson and Lewandowski 1996; NACE 2006).

The detection of iron- and manganese-oxidizing bacteria is usually dependent on diagnostic liquid cultures, which is very difficult even for experienced microbiologists. Microscopic identification of iron-oxidizing bacteria is also quite difficult for an experienced analyst. Several direct and indirect tests for the presence of corrosion-causing bacteria are summarized in NACE Standard TM0101-2006 (NACE 2006). However, these techniques are not

capable of quantifying metal-oxidizing bacteria. A new technique called quantitative polymerase chain reaction (qPCR) is now available for quick detection and quantification by targeting 16S rRNA gene of *Leptothrix*, *Sphaerotilus*, and *Gallionella* (Zhu, Ayala et al. 2005; Zhu, Modi et al. 2006). The presence of iron-oxidizing bacteria within tubercles associated with localized corrosion is considered a positive indication of MIC.

1.1.1.1.4 MRB-induced corrosion

Under oxic conditions, the metal surface becomes oxidized, causing the formation of metal oxides and hydroxides, which protect the metal surface from further corrosion. Some metal-reducing bacteria (MRB) are capable of using metal oxides or hydroxides (Fe^{3+} and Mn^{4+}) as electron acceptors efficiently (i.e., redox potential is similar to nitrate) and out-compete low potential electron acceptors such as sulfate or carbon dioxide (Myers and Nealson 1988). When MRB is in direct contact with solid iron (Fe^{3+}) and manganese (Mn^{4+}) oxides, the dissimilatory reduction produces soluble ions (Fe^{2+} and Mn^{2+}), resulting in dissolution of surface oxides. This destabilizes the passivating protective film (oxide film) and allows further corrosion (localized corrosion) to take place (Little and Wagner 2001; Horn and Jones 2002). Medium containing ferric citrate ($FeC_6H_5O_7 \cdot 3H_2O$) as the terminal electron acceptor and acetate as the sole carbon source can be used to detect the presence of IOB. A positive indication of growth and iron reduction is a color change in the medium from brown to green (NACE 2006).

1.1.1.1.5 Other bacteria-induced corrosion

Acidophilic sulfur/sulfide-oxidizing bacteria oxidize sulfide or elemental sulfur to sulfate or sulfuric acid. For example, *Thiobacillus* bacteria are the most common sulfur-oxidizing bacteria, and are almost always accompanied by SRB. Sulfur/sulfide-oxidizing bacteria obtain the carbon required for the synthesis of new cell material by fixation of CO_2 from the atmosphere and energy from oxidation and reduction reactions (Little, Ray et al. 2000; Dubey and Upadhyay 2001). Ferrous iron from reduced sulfur compounds serve as the electron donor, and oxygen is the preferred electron acceptor. In the absence of oxygen, organisms grow on reduced inorganic sulfur compounds using ferric iron as an alternative electron acceptor. The specific oxidation reactions leading to production of sulfuric acid (H_2SO_4) varies with the initial reduced sulfur species (H_2S, $S_2O_3^{2-}$, $S_3O_6^{2-}$, $S_4O_6^{2-}$, S^0). Elemental sulfur, thiosulfates, metal sulfides, H_2S, and tetrathionates can be oxidized to H_2SO_4 (Nordstrom and Southam 1997).

Methanogens and some strains of SRB frequently co-exist in a symbiotic relationship. They remove hydrogen from the surface of metals catalyzed by a reversible hydrogenase, enhance the cathodic reduction of proton (cathodic depolarization), and thereby accelerate anodic metal dissolution (Boivin, Laishley et al. 1990; Horn and Jones 2002). Culturing of methanogens is very difficult due to the strictly anaerobic nature of methanogens. Genetic techniques are now available for quick detection and quantification of methanogens by targeting a specific functional gene (Zhu, Modi et al. 2006).

Nitrate- and nitrite-reducing bacteria use nitrogen oxides as alternative electron acceptors under anoxic conditions (Braker, Fesefeldt et al. 1998). In the presence of nitrate, denitrifying bacteria are reported to cause metal corrosion (Kholodenko 2000; Nemati, Jenneman et al. 2001).

Hydrogen embrittlement of metals occurs when molecular hydrogen invades the metal lattice, filling interstitial regions and thereby distorting the lattice structure and weakening the metal-metal bond (Horn and Jones 2002). Bacterial production of hydrogen can directly promote hydrogen embrittlement of metals. Indirectly, the generation of acids, which can be reduced to hydrogen at cathodic sites, as well as the generation of sulfide, which promotes the adsorption of hydrogen into metal matrices may also promote hydrogen embrittlement.

MIC Detection and Monitoring

Internal MIC is a significant problem affecting the oil, gas and other industries. Routine monitoring of water quality may identify potential problem organisms and the factors that may promote bacterial growth and attack. Water quality parameters that are considered important to understanding internal corrosion and MIC for a particular industrial system include temperature, pH, alkalinity, sulfide, nitrite, dissolved gases (CO_2, H_2S, O_2, NH_3, etc.), total dissolved solid (TDS), chemical oxygen demand (COD), microorganisms (bacteria, algae, and fungi), etc. COD measures the concentration of electron donors available for sulfate or metal reduction; hence a low COD means a low risk of finding SRB and iron-reducing bacteria in the system. On the other hand, dissolved oxygen might not be indicative as to the oxygen content within the biofilm. Nevertheless, changes in these parameters, especially long-term trends in one direction or large anomalies, indicate a need for further investigation. Online monitors are commercially available for monitoring temperature, pH, conductivity, and TDS, and portable or laboratory spectrophotometers and kits are available for the other tests. MIC investigations require microbiological, chemical, and metallurgical testing for proper diagnosis.

Free-floating planktonic bacteria are often the focus of monitoring for MIC since system fluids are generally easier to sample than metallic surface. However, the results of planktonic bacteria can sometimes be misleading as to whether MIC will occur or, if so, to what extent (NACE; Zintel, Licina et al. 2001). Many bacteria such as *Pseudomonas*, *Serratia*, and SRB secrete EPS, which improves the adherence capacity to a metal surface and promotes further trapping of microorganisms in the substratum. The environmental conditions at biofilm/surface interfaces are often radically different from the bulk medium in terms of pH, dissolved oxygen, and other organic and inorganic species. Oxygen consumption by aerobic bacteria living in the surface region of the biofilm leads to the creation of an anaerobic space for the growth of anaerobic bacteria, which, in turn, results in the formation of oxygen concentration gradients and differential aeration cell on a metal surface (Tanji, Itoh et al. 2002). The most devastating MIC takes place in the presence of microbial consortia in which many physiological types of bacteria, including SRB, APB, MOB and MRB, interact in a complex way within the structure of biofilms (Little, Wager et al. 1991; Pope and Pope 1998; Le Borgne, Romero et al. 2007). Comparing to planktonic bacterial counts, sessile bacteria (e.g., biofilm) are more relevant to microbial corrosion (Hernández-Gayosso, Zavala-Olivares et al. 2004). However, monitoring sessile bacteria or biofilm is more complicated, requiring either that the pipeline be excavated or halted for internal sampling or that accommodations be made in the system design to allow for regular collection or on-line tracking of attached organisms during operation.

The most commonly used means of monitoring MIC is to quantify the number of bacteria capable of growing in various microbial growth media (solid or liquid) after inoculation with water samples (serial dilution) obtained from pipelines and other locations (NACE; API 1982).

Solid samples such as internal deposits, corrosion products, and surface swabs should be suspended in a sterile phosphate buffer to release viable microbes for inoculation. After incubation at certain temperature for a pre-determined period of time (days to weeks), the result is expressed as the number of colony forming units (CFU) for solid medium or the most probable number (MPN) for liquid medium. Many bacteria growth media are commercially available or can be made in the laboratory to selectively grow and detect certain type of microbes – aerobic bacteria, anaerobic bacteria, APB, SRB, sulfur-oxidizing bacteria, iron-related bacteria, low nutrient bacteria, nitrite/nitrate-reducing bacteria, and slime-forming bacteria, fungi, algae, etc. General aerobic or anaerobic bacteria counts are normally always included in a MIC monitoring program to gauge the environmental conditions for microbial growth. Some microorganisms such as sulfur-oxidizing bacteria, iron-oxidizing bacteria, and iron-reducing bacteria are very difficult to grow in culture, and the indicators for active growth sometimes are not always appropriate or easy to identify. It is also important to note that the bacterial growth media that are intended to support the growth of a particular type of bacteria are not completely selective, and the vast majority (90-99%) of microbial species cannot currently be grown in the laboratory (Muyzer, de Waal et al. 1993; Williams, Lobos et al. 1997; Maidak, Cole et al. 2001; Zhu 2002), thus underestimating the size and misrepresenting the true composition of microbial communities in the sample (Torsvik, Goksoyr et al. 1990; Osburne 2000; Zhu, Ayala et al. 2005).

Correct and consistent procedures are crucial for the success of growth methods in MIC monitoring. Sample collection may expose microorganisms to abrupt changes in pressure, temperature, atmosphere, and light, causing redistribution in numbers and types of microorganisms in the original samples. Therefore, the sample collection method, sample transportation, culturing techniques and growth medium, incubation temperature and time should be kept strictly controlled in order to reveal trends in bacteria number over long periods of time. This information is far more important and useful than a single data point when detecting and monitoring the microbial corrosion in a particular system. NACE Standard TM0194-2004 details the sampling procedures for planktonic bacteria, culturing techniques, growth medium and growth indicator for general heterotrophic bacteria and SRB, and provides the guidelines for the assessment of sessile bacteria (NACE).

To circumvent problems associated with cultivation-based methods, many culture-independent genetic techniques have been developed in the past decade (Harmsen, Akkermans et al. 1996; Zhu and Joerger 2003), and are beginning to be used in the oil and gas industry for problems related to MIC. One such method is called reverse sample genome probing (RSGP), which allows determination of up to 30 SRB species on an environmental sample in a single DNA hybridization assay (Voordouw, Jack et al. 1994; Telang 1997; Voordouw 1998). Another example of genetic method is quantitative polymerase chain reaction (qPCR) (Suzuki, Taylor et al. 2000; Stults 2001; Skovhus, Ramsing et al. 2004; Zhu, Modi et al. 2006). qPCR can be designed to target and quantify a specific gene which only exists in a specific species or specific group of bacteria, such as SRB, APB and IOB. qPCR has also been used to determine microorganism abundance in many different types of complex environmental samples such as sediments, water, wastewater, feces, and marine samples, from domain down to genus and species levels (Suzuki, Taylor et al. 2000; Stults 2001; Ibekwe, Watt et al. 2002; Brinkman, Haugland et al. 2003; Guy, Payment et al. 2003). The results are more accurate and can be

obtained in a few hours instead of days or weeks required for traditional growth methods (Zhu, Ayala et al. 2005; Zhu, Modi et al. 2006). Unlike traditional culturing method, qPCR detects and quantifies the target microorganisms in the samples without cultivation, thus, it does not alter the composition of the microbial community in the original sample. In addition, qPCR also works for dry and old samples without live bacteria, a huge advantage over traditional growth methods.

Bacteria in the water sample can also be directly counted under a microscope with or without staining. With proper staining (e.g., fluorescent dye), it is even possible to distinguish the live and dead bacteria under microscope. If bacteria are stained with fluorescently labeled oligonucleotides, it is possible to identify the genera or species of microbes in microbial communities, helping understand how biofilms develop and influence corrosion processes. However, direct counting with a microscope is difficult, time consuming, and sometimes impossible when the sample is turbid or colored, and requires a well-trained observer to gain useful information. Hydrocarbon, deposits, and other contaminants in the sample occasionally fluoresce under ultraviolet light thereby preventing the use of fluorescent dye. Other enumeration methods involve the measurement of molecules peculiar to microbes (e.g., antibody-based SRB enumeration), or biochemical activities (e.g., hydrogenase-based SRB enumeration, adenosine triphosphate or ATP assay). These methods are generally difficult to calibrate against "real world" microbes and have high detection limits.

Chemical characterization of corrosion products and bulk fluids collected from corrosion sites is also important in the diagnosis of MIC. Inductively coupled plasma atomic emission spectroscopy (ICP-AES), ion chromatography (IC), and other traditional colorimetric and spectrophotometric assays are commonly used to measure elemental concentrations in water or pipeline deposit samples. Metallurgical testing includes energy-dispersive X-ray (EDX), X-ray diffraction (XRD), and Raman spectroscopy. These are used to analyze corrosion morphology (pitting depth, shape, coverage, etc.) and corrosion products (chemical composition, compounds, etc.). Other techniques such as scanning electron microscope (SEM), environmental SEM (ESEM), and confocal scanning laser microscope can also be used to qualitatively evaluate the biofilm and/or corrosion products (Horn and Jones 2002). The integrated consideration of chemical and metallurgical data, microbial data and operational conditions is needed for proper detection and diagnosis of MIC (Horn and Jones 2002).

The choice of internal corrosion (including MIC) monitoring is based on a variety of factors, such as leak history, product quality, presence of corrosion indicators detected in previous samples (e.g., dew point and/or free water levels, acid gas pressures, iron, and bacteria counts, etc.), as well as other operational and economic factors. In many oil and gas operations, monitoring has often combined with the use of corrosion detection devices with sampling and analysis of gas, liquids, and solids obtained from the system. Under some conditions, microbial corrosion and overall internal corrosion may be monitored using corrosion coupons or probes. The coupons are made from an alloy similar to the metal in the system, and typically installed in the bottom quadrant of gas lines so they would be exposed to any liquids that condensed or are inadvertently put into the system, or in a "side-stream" which offers the additional advantage of allowing one to experimentally alter biocide levels and process conditions, giving reasonably fast and reliable information on their affects on the system. The presence of biofilm and microbial activities on a coupon surface change the local chemistry, possibly modifying the local anodic

and cathodic processes and initiating or dramatically altering corrosion process such as pitting. Extensive microscopic analysis of coupons can yield important evidence with regard to pit initiation mechanisms, identify the severity of localized attack through the measurement of pitting (pit densities, depths, and diameters), calculate pitting rates by bacteria or other corrosive components, and determine the severity of attack.

The drawback of corrosion (including MIC) monitoring with metal coupons or probes is that it is destructive and requires time-consuming analysis of numerous coupons sequentially placed in the pipeline in order to obtain information on long-term buildup of biofilms and corrosion initiation. Various electrochemical techniques have been developed for nondestructive and long-term monitoring of the formation and activity of biofilm and possibly detection of an early MIC problem (Blackburn; Dorsey, Licina et al. 2002; Hernández-Gayosso, Zavala-Olivares et al. 2004). Such electrochemical techniques include electrical resistance (ER) probes, linear polarization resistance (LPR) probes, galvanic probes, hydrogen probes, electrochemical impedance spectroscopy (EIS), electrochemical noise (ECN), etc. ER probes are used to determine metal loss by measuring the increase in resistance of a metal specimen as its cross-sectional area is reduced by corrosion. LPR probes measure instantaneous corrosion rates and qualitative pitting tendency of metals in electrolytes. ECN measures the fluctuations of the potential, current and resistance over time and then determines the overall corrosion rates and rapid sustained pitting (RSP). For example, Hernández-Gayosso and colleagues successfully detected the formation of biofilm, increased corrosion rate and initiation of localized corrosion on electrodes using EIS technology (Hernández-Gayosso, Zavala-Olivares et al. 2004).

One drawback to most electrochemical techniques is the need for electrolytes in the area of the measuring device. Another weakness of most electrochemical techniques is the failure to quantify the localized corrosion, especially RSP (Pope 1992; Royer and Unz 2002). These techniques give average readings for the surface of a test electrode, and it is not clear whether a measured corrosion current corresponds to uniform corrosion of the entire surface or to localized corrosion of just a few sites on the surface. In the latter case, corrosion rates will be severely underestimated if the measured corrosion loss is not normalized to the area at which localized corrosion occurs. This general disadvantage of electrochemical techniques is especially bothersome in the case of MIC, where most corrosion processes are of an extremely localized nature (Little and Wagner 2001; Horn and Jones 2002).

MIC Prevention and Mitigation

Once internal MIC has been established in a pipeline, complete mitigation is neither practical nor possible. Therefore, the prevention of internal MIC from being initially established should be a top priority. One of the first defense systems against internal corrosion is to ensure that the product being transported is free of moisture. For corrosion to occur, there must be moisture, CO_2, O_2, or some other reduction reactant, such as one produced by microbes. Gathering lines in production fields have a much more significant problem with internal corrosion than the typical transmission pipeline. MIC after hydrotesting is a common problem when the system was not completely dried after testing. Water used in hydrotesting should be as clean as possible by removing particulates, contaminants and nutrients such as oils, iron, phosphate, and nitrate. When necessary, water should be treated to reduce hardness, remove oxygen, or alter pH.

Although coatings/linings have been used on the internal aspects of natural gas pipelines principally to improve flow characteristics, some internal linings also appear to protect against at least some forms of corrosion, including MIC, by effectively isolating the pipeline from the impact of surrounding environment (Lockwood, Paakkonen et al. 1993). However, due to its feasibility and cost, internal coatings are generally limited to new installations or areas easily accessible to "in situ" lining and areas in which pigging would not destroy the integrity of the lining. It should be noted that the target area must be completely lined. Failure to coat weld regions or other features in contact with lined portions of the system could focus corrosion on the unlined areas, thereby accelerating corrosion in these areas. In addition, coating performance can be compromised by microbial degradation of coatings or components in the coating system, leading to water permeation and disbondment of coating. MIC regularly takes place on pipe surfaces under the disbonded coatings, where water and nutrients promote the growth of microorganisms, resulting in the formation of corrosion cells. The severity of corrosion under the disbonded coating strongly depends on the conductivity of the water trapped in the pocket under the separated coating.

System design, maintenance, and water quality are the keys to MIC prevention and control (Lutey 1993; Scott 2004). Materials selection, accessibility for cleaning and water treatment, provision for drains, traps, recycle circuits, and monitoring equipment, control of water velocity and elimination of stagnant, low-flow areas and dead legs, and minimization of crevices and welds are the key considerations in system design. Regular cleaning, including chemical and mechanical cleaning, should be part of the operating routine to remove sludge, deposits, and foulants from the system.

The mitigation measures of internal MIC consist primarily of mechanical cleaning (pigging) and chemical treatment (biocides and corrosion inhibitors). Chemical treatments usually involve the use (in batch or continuously) of biocides, corrosion inhibitors or both to control microbes in the system. A successful MIC control program requires assessment of the MIC potential in a system, screening tests of chemical treatments, and aggressive monitoring of actual systems after treatment. It is worth noting that most laboratory studies of biocide efficiency in man-made system often fail to duplicate their successful results when they are applied in industrial systems. Organisms embedded within the biofilm are protected from biocides, largely due to the diffusion barriers generated by the EPS matrix that hinders the chemical penetration of the entire thickness of the deposits (Nichols, Evans et al. 1989; Batista 2000). Moreover, bacteria within the biofilm are probably physiologically altered and may develop resistance to a particular biocide if it is used repeatedly (Kajdasz, Einstman et al. 1984; Farthing 1997). Therefore, before the biocide treatment, a “time-kill” study is often needed to identify what chemical agent(s) is (are) the most effective in killing the bacteria in a particular system.

The resistance of bacteria to biocides depends on the nature of the chemicals used. Biocides can be classified as either oxidizing or non-oxidizing. Apart from ozone and hydrogen peroxide, all the oxidizing agents used as biocides contain halogens. The non-oxidizers are relatively non-reactive chemicals and, therefore, compatible with strong reducing agents in water treatment application (McCoy 1987). Examples of typical non-oxidizing biocides are formaldehyde, glutaraldehyde, methanol, isothiazolones, quaternary amines, and tetrakishydroxymethylphosphonium sulfate (THPS). Non-oxidizing biocides are often used in

combination with dispersants and surfactants to stimulate full biocides penetration into the biofilm. Whether biocides can be used continuously or in a batch mode, or periodically depends on the system. In the case of continuous treatment, it is necessary to alternate several biocides to prevent biocide resistant bacteria strain from being developed. Batch treatment is usually applied to the system after hydrotesting and pigging operation. The effectiveness of biocide treatments depend on proper treatment schedule, effective doses, and appropriate locations, and combination with other control technologies (e.g., pigging) (Lockwood, Paakkonen et al. 1993). For instance, an additional pigging run using a sphere or ball pig to push a slug of a biocide solution (1% cocodiamine and quaternary in methanol) was reported to be very effective to keep the pipe free of bacteria after hydrotesting (Farthing 1997). The mixture biocide solution in this treatment also acts as a corrosion inhibitor against carbon dioxide and hydrogen sulfide attack.

Batch or continuous injection of corrosion inhibitors is also commonly employed to treat/prevent many types of corrosion including MIC. Most corrosion inhibitors used in the natural gas industry are more effective in preventing and treating generalized-type corrosion than the focused, RSP corrosion usually associated with MIC, due to the difficulty in penetrating existing biofilms and corrosion products and to the fact that bacteria may degrade some corrosion inhibitors (Pope, Zintel et al. 1989; Pope and Skultety 1995). The concentrations of biocides and corrosion inhibitors have to be closely monitored in the system during treatment since the treatment chemicals can be degraded or used up faster by factors such as pH, TDS, chlorides, temperature, oxygen, etc. Spore-forming microorganisms such as species in genus *Bacillus* and *Clostridium* can usually survive biocide treatment, and re-generate in the pipeline system when biocide concentration becomes lower and other conditions become favorable. *Bacillus* has been isolated frequently from tubercles formed on metals and associated with microfouling (de Franca and Lutterbach 1996). These organisms are copious producers of organic acids.

"Pigs" are the most common device used for the mechanical cleaning of the pipeline interior, and pigging is one of the most effective means of controlling microbes on metal surfaces and, therefore, internal MIC. Pigs are inserted into the pipelines and pushed through the pipe using gas pressure. The frequency of pigging and types of pigs utilized are determined, at least in part, by the results of the pigging itself, such as the amount and types of materials removed from the line. The objectives of mechanical cleaning are to remove materials capable of inhibiting gas flow and/or promoting corrosion (including MIC) from the pipeline. These materials include fluids (including water) and solids (e.g., sand, corrosion products, nodules, and biofilms/slimes). Water is required for microbial metabolism and growth and corrosion processes; solids provide shelter for microorganisms and water, reduce the efficiency of treatment chemicals (e.g., biocides and corrosion inhibitors), and allow the formation of concentration cells.

In addition to viable microbes in the removed materials, pH, iron, chloride, and sulfide should also be measured in the monitoring program. Chloride (Cl^-) ions are very aggressive and participate in many forms of corrosion, including MIC. Chloride ions from the electrolyte migrate to the anode to neutralize any buildup of charge, forming heavy metal chlorides that are extremely corrosive to metal surface, particularly stainless steels. Under these circumstances, pitting involves the conventional features of differential aeration, a large cathode-to-anode surface area, and the development of acidity and metallic chlorides (NACE 2006). Webster and

Newman examined the impact of media constituents on localized corrosion and concluded that localized corrosion would not readily occur unless chloride ion was the predominant anion in the medium (Webster and Newman 1994). Sulfide levels in the corrosion products and fluids can serve as an indication of MIC-type corrosion.

A very different approach which has been proposed as a potential alternative to protect pipeline from internal corrosion is to use beneficial biofilm on metal surface as a corrosion inhibition mechanism (Little and Ray 2002). Biofilms have been reported to be effective on inhibition of general corrosion in some circumstance for mild steel, copper, aluminum, and stainless steels (Jayaraman, Hallock et al. 1999; Jayaraman, Ornek et al. 1999; Chan, Xu et al. 2002; Zuo, Ornek et al. 2004; Zuo and Wood 2004; Zuo 2007). The mechanisms most frequently cited for the inhibition are:

1) formation of a diffusion barrier to corrosion products that stifles metal dissolution,
2) removal of corrosive agents (e.g., oxygen) from metal surface by bacteria physiological activities (e.g., aerobic respiration) (Hernandez, Kucera et al. 1994; Jayaraman, Cheng et al. 1997),
3) growth inhibition of corrosion-causing bacteria by antimicrobials generated within biofilm (e.g., SRB corrosion inhibition by gramicidin S-producing *Bacillus brevis* biofilm (Jayaraman, Hallock et al. 1999; Zuo, Ornek et al. 2004; Zuo and Wood 2004),
4) generation of protective layer by biofilms (e.g., *Bacillus licheniformis* biofilm produces on aluminum surface a sticky protective layer of gamma-polyglutamate) (Hernandez, Kucera et al. 1994),
5) formation of passive layers (e.g. magnetite film) (Hernandez, Kucera et al. 1994), and
6) production of metabolic products that act as corrosion inhibitors (e.g., siderophores) (McCafferty and McArdle 1992; Eashwar and Maruthamuthu 1995).

However, biofilm formation on metal surface is unpredictable and uncontrollable, and is often not uniform. Bacteria tend to colonize preferentially on rough surfaces and are more attracted to anodic sites (Little, Wagner et al. 1991). Biofilm growth rate depends on substratum, available nutrients, temperature, and electron acceptors. Biofilm composition is affected by small perturbations in the environment (e.g., temperature, nutrient concentration, and flow). A little-understood phenomenon – biofilm sloughing – creates a discontinuity of biofilm on metal surface (patchiness), which results in local differences in metabolic products, pH, dissolved oxygen, and gradients of nutrients and ions within the biofilm. Patchy biofilms create differential aeration cells which can lead to intensification of localized corrosion rates under the biofilms (Geesey 1991; Heitz 1996). Biofilm formation is an extremely complex biological/chemical process, and its impact on corrosion processes is difficult to predict and control. Therefore, more research is needed before biofilms can be used as corrosion inhibition mechanisms in the field.

1.1.2 *Task 1 – Literature Review/Collection of biogas/biomethane Chemical Data*

The center piece of this research is the need to understand the integrity impacts of transporting various biogas/biomethane products through existing non-metallic pipelines and its non-metallic components. To aid in this investigation it is necessary to fully comprehend the composition of potential fuel gases that may come into contact with these materials.

One phase of task one includes the collection of analytical data from biogas and biomethane derived from dairy manure, landfills, and wastewater treatment plants (WWTP). Data was obtained from previous GTI projects as well as samples sent to the analytical laboratory from industrial customers. In addition, an extensive literature review was performed and several datasets from biogas facilities were obtained. This data will be compared with comprehensive natural gas data sets acquired over the past two decades.

Consistent with GTI's previous research projects, constituents of the biogas or biomethane were categorized into two tiers. The *First Tier* includes compounds that are consistent with those found in natural gas pipeline tariffs. *Second Tier* target compounds include compounds of concern which are not routinely monitored in natural gas but are a potential hazard to both human health and infrastructure. Table 2 and Table 3 include target compounds from the First Tier grouped based on analytical methods used for detection and quantification. Table 4 and Table 5Table 5 include target compounds from the Second Tier and is also grouped based on analytical methods used for detection and quantification. Not all samples were subject to all analyses. Data collected from literature review and samples from industrial customers provided data for biogas of only the First Tier compound list; however, only a partial list of compounds was acquired for some samples.

GTI project *Pipeline Quality Biomethane: North American Guidance Document for Introduction of Dairy Waste Derived Biomethane into Existing Natural Gas Networks* evaluated biogas and biomethane from dairy farms from three geographic regions in the United States. Samples from 14 different farms producing biogas and/or biomethane were collected and analyzed for all chemical groups in the first and second tier. Twelve samples of raw biogas were collected from 12 different farms. Another 8 samples were collected from 5 biogas facilities that provided partial upgrading. A total of 23 samples from 2 biogas facilities that executed full upgrading to biomethane were collected.

As part of GTI's *Pipeline Quality Biogas: Guidance Document for Dairy Waste, Wastewater Treatment Sludge and Landfill Conversion* (PHMSA Project 250), a total of 51 samples were analyzed for all compound groups in the first and second tier. This includes 16 biomethane samples from landfill gas and 5 biomethane samples from WWTPs, 14 biogas samples from landfill and 5 from WWTPs, and 11 natural gas samples.

GTI's analytical laboratory provides a variety of analytical services for the natural gas and fuels-related industry. Many industrial customers send samples to GTI for analysis of biogas. In the past four years, a total of 341 biogas samples were analyzed by the laboratory for first tier chemical groups; 42 from dairy farms, 166 from landfills, and 133 from wastewater treatment facilities. In addition, 24 natural gas trace constituent samples were obtained.

Previous GTI (formerly GRI and IGT) projects evaluated trace constituents in natural gas from across the United States and Canada. The projects provide analytical data from the first tier chemical group. A total of 29 samples were obtained for review for this project. Analytical data for natural gas samples from these projects and the PHMSA project will be compiled into table format and presented in the next quarterly update.

A literature review of existing analytical data from biogas and biomethane yielded 71 samples from 5 reports; 46 biogas samples from landfill, 5 biogas samples from dairy and 20 biogas samples from WWTP. However, data from these reports present data only on major components of biogas and limited data on volatile organic compounds (VOCs) and siloxanes.

The data collected from previous GTI projects, GTI analytical laboratory results from industrial customers, and literature search results have been complied into table format and is included in the Appendices. Appendices A- C includes *First Tier* compounds from biogas, biomethane and natural gas, respectively. Appendices D-F includes *Second Tier* compounds from biogas, biomethane and natural gas, respectively.

Table 6 summarizes the number of samples collected for review based on type of gas and source of information. During the next quarter, the data from all sources will be organized into a consistent table format for easier review.

Table 2. Target Compounds for First Tier Chemical Testing, Part A

Halocarbons	Siloxanes	Metals
Dichlorodifluoromethane (CFC-12)	1,1,3,3-Tetramethyldisiloxane	Arsenic
1,2-Dichlorotetrafluoroethane (CFC-114)	Pentamethyldisiloxane	Barium
1,1,2-Trichloro-1,2,2-trifluoroethane (CFC-113)	Hexamethyldisilane	Beryllium
Trichlorofluoromethane (CFC-11)	Hexamethyldisiloxane	Cadmium
Chloromethane	Octamethyltrisiloxane	Cobalt
Dichloromethane (Methylene Chloride)	Octamethylcyclotetrasiloxane	Chromium
Chloroform	Decamethyltetrasiloxane	Copper
Carbon Tetrachloride	Decamethylcyclopentasiloxane	Manganese
Chloroethane	Dodecamethylpentasiloxane	Molybdenum
1,1-Dichloroethane		Nickel
1,2-Dichloroethane		Lead
1,1,1-Trichloroethane		Antinomy
1,1,2-Trichloroethane		Selenium
1,1,2,2-Tetrachloroethane		Strontium
Chloroethene (Vinyl Chloride)		Thallium
1,1-Dichloroethene		Zinc
cis-1,2-Dichloroethene		
Trichloroethene		
Tetrachloroethene		
1,2-Dichloropropane		
3-Chloropropene		
cis-1,3-Dichloropropene		
trans-1,3-Dichloropropene		
Bromomethane		
1,2-Dibromoethane		
Chlorobenzene		
1,2-Dichlorobenzene		
1,3-Dichlorobenzene		
1,4-Dichlorobenzene		
1,2,4-Trichlorobenzene		
Hexachloro-1,3-butadiene		

Table 3. Target Compounds for First Tier Chemical Testing, Part B

Major Components	Extended Hydrocarbons	Sulfur Compounds	Calculated Real Gas Properties
Helium	Cyclopentane	Hydrogen Sulfide	Compressibility Factor
Hydrogen	Methylcyclopentane	Sulfur Dioxide	Specific Gravity
Carbon Dioxide	Cyclohexane	Carbonyl Sulfide	Gross HV (Btu/ft^3)
Oxygen/Argon	Methylcyclohexane	Carbon Disulfide	Wobbe Index
Nitrogen	Benzene	Methyl Mercaptan	Net HV (Btu/ft^3)
Carbon Monoxide	Toluene	Ethyl Mercaptan	Density
Methane	Ethylbenzene	i-Propyl Mercaptan	
Ethane	m,p-Xylene	n-Propyl Mercaptan	
Ethene	Styrene	t-Butyl Mercaptan	
Ethyne	o-Xylene	Dimethyl Sulfide	
Propane	C3 Benzenes	Methyl Ethyl Sulfide	
Propene	Naphthalene	Diethyl Sulfide	
Propadiene	C1 Naphthalenes	Di-t-Butyl Sulfide	
Propyne	C2 Naphthalenes	Dimethyl Disulfide	
i-Butane	Hexanes	Methyl Ethyl Disulfide	
n-Butane	Heptanes	Methyl i-Propyl Disulfide	
1-Butene	2,2,4-Trimethylpentane	Diethyl Disulfide	
i-Butene	Octanes	Methyl n-Propyl Disulfide	
trans-2-Butene	Nonanes	Methyl t-Butyl Disulfide	
cis-2-Butene	Decanes	Ethyl i-Propyl Disulfide	
1,3-Butadiene	Undecanes	Ethyl n-Propyl Disulfide	
i-Pentane	Dodecanes	Ethyl t-Butyl Disulfide	
n-Pentane	Tridecanes	Di-i-Propyl Disulfide	
neo-Pentane	Tetradecanes	i-Propyl n-Propyl Disulfide	
Pentenes	Pentadecanes	Di-n-Propyl Disulfide	
Hexane Plus	Hexadecanes	i-Propyl t-Butyl Disulfide	
Ammonia	Heptadecanes	n-Propyl t-Butyl Disulfide	
	Octadecanes	Di-t-Butyl Disulfide	
	Nonadecanes	Dimethyl Trisulfide	
	Eicosanes +	Diethyl Trisulfide	
		Di-t-Butyl Trisulfide	
		Thiophene	
		C1-Thiophenes	
		C2-Thiophenes	
		C3-Thiophenes	
		Benzothiophene	
		C1-Benzothiophenes	
		C2-Benzothiophenes	
		Thiophane	
		Thiophenol	

Table 4. Target Compounds for Second Tier Chemical Testing, Part A

Semi-volatile/Volatile Organic Compounds			Aldehydes/ Ketones
1,1,1-Trichloroethane	2-Chlorophenol	2,6-dinitrotoluene	Formaldehyde
1,2-Dichloroethane	1,3-Dichlorobenzene	1,2-Dinitrobenzene	Acetaldehyde
1,1-Dichloropropene	1,4-Dichlorobenzene	3-Nitroaniline	o-Tolualdehyde
Benzene	p-Isopropyltoluene	Acenaphthene	Acetone
Carbon Tetrachloride	Benzyl Alcohol	2,4-Dinitrophenol	Isocaleraldehyde
1,2-Dichloropropane	2-Methylphenol (m-cresol)	4-Nitrophenol	Valeraldehyde
Trichloroethene	1,2-Dichlorobenzene	Dibenzofuran	Butyraldehyde
Dibromomethane	3,4-Methylphenol	2,4-dinitrotoluene	m-Tolualdehyde
Bromodichloromethane	bis(2-chloroisopropyl)ether	2,3,4,6-Tetrachlorophenol	Propionaldehyde
Pyridine	n-Butylbenzene	2,3,5,6-Tetrachlorophenol	Crotonaldehyde
cis-1,3-Dichloropropene	N-nitroso-di-n-propylamine	Diethylphthalate	2,5-Dimethyl-
N-nitrosodimethylamine	Hexachloroethane	4-Chlorophenyl-phenylether	benzaldehyde
Toluene	1,2-Dibromo-3-	Fluorene	Benzaldehyde
trans-1,3-Dichloropropene	Chloropropane	4-Nitroaniline	p-Tolualdehyde
1,1,2-Trichloroethane	Nitrobenzene	4,6-Dinitro-2-methylphenol	Hexanaldehdye
1,3-Dichloropropane	Isophorone	n-Nitrosodiphenylamine	Methyl ethyl
Dibromochloromethane	2-Nitrophenol	4-Bromophenyl phenyl	ketone
1,2-Dibromoethane	2,4-Dimethylphenol	ether	
Tetrachloroethene	bis(2-Chloroethoxy)	Hexachlorobenzene	
Chlorobenzene	methane	Pentachlorophenol	
1,1,1,2-Tetrachloroethane	1,2,4-Trichlorobenzene	Phenanthrene	
Ethylbenzene	Naphthalene	Anthracene	
m/p-Xylenes	2,4-Dichlorophenol	Carbazole	
Bromoform	4-Chloroaniline	Di-n-butylphthalate	
Styrene	Hexachlorobutadiene	Bis(2-ethylhexyl) adipate	
o-Xylene	1,2,3-Trichlorobenzene	Fluoranthene	
1,1,2,2-Tetrachloroethane	4-Chloro-3-methylphenol	Pyrene	
1,2,3-Trichloropropane	2-Methylnaphthalene	Butylbenzylphthalate	
Isopropylbenzene	1-Methylnaphthalene	Benz[a]anthracene	
Bromobenzene	Hexachlorocyclopentadiene	Chrysene	
2-Chlorotoluene	2,4,6-Trichlorophenol	bis(2-Ethylhexyl)phthalate	
n-Propylbenzene	2,4,5-Trichlorophenol	Di-n-octylphthalate	
4-Chlorotoluene	Diphenylamine	Benzo[b]fluoranthene	
1,3,5-Trimethylbenzene	Azobenzene	Benzo[k]fluoranthene	
tert-Butylbenzene	2-Chloronaphthalene	Benzo[a]pyrene	
1,2,4-Trimethylbenzene	2-Nitroaniline	Indeno[1,2,3-cd]pyrene	
sec-Butylbenzene	1,4-Dinitrobenzene	Dibenz[a,h]anthracene	
Phenol	Dimethylphthalate	Benzo[g,h,i]perylene	
bis(2-Chloroethyl)ether	1,3-Dinitrobenzene		
Aniline	Acenaphthylene		

Table 5. Target Compounds for Second Tier Chemical Testing, Part B

Pesticides	Polychlorinated Biphenyls				Pharmaceuticals
a-BHC	PCB 2	PCB 73	PCB 81	PCB 158	Ampicillin Trihydrate
b-BHC	PCB 3	PCB 49	PCB 87	PCB 129	Amoxicillin Trihydrate
g-BHC	PCB 4	PCB 47	PCB 115	PCB 178	Oxytocin
d-BHC	PCB 10	PCB 48	PCB 85	PCB 175	Florfenicol
Heptachlor	PCB 7	PCB 75	PCB 136	PCB 187	Ceftiofur
Aldrin	PCB 9	PCB 104	PCB 77	PCB 183	Tilmicosin
Heptachlor epoxide	PCB 6	PCB 35	PCB 110	PCB 128	Furosemide
g-Chlordane	PCB 8	PCB 44	PCB 154	PCB 167	Flunixin meglumine
Endosulfan I	PCB 5	PCB 59	PCB 82	PCB 185	Fenbendazol
a-Chlordane	PCB 19	PCB 37	PCB 151	PCB 174	Doramectin
Dieldrin	PCB 12	PCB 42	PCB 135	PCB 177	Tripelennamine
4,4'-DDE	PCB 13	PCB 71	PCB 144	PCB 202	hydrochloride
Endrin	PCB 18	PCB 41	PCB 124	PCB 171	
Endosulfan II	PCB 17	PCB 64	PCB 147	PCB 156	
4,4'-DDD	PCB 15	PCB 40	PCB 107	PCB 173	
Endrin aldehyde	PCB 24	PCB 103	PCB 123	PCB 157	
Endosulfan sulfate	PCB 27	PCB 67	PCB 149	PCB 201	
4,4'-DDT	PCB 16	PCB 100	PCB 118	PCB 172	
Endrin ketone	PCB 32	PCB 63	PCB 134	PCB 197	
Methoxychlor	PCB 34	PCB 74	PCB 114	PCB 180	
	PCB 29	PCB 70	PCB 131	PCB 193	
	PCB 54	PCB 66	PCB 122	PCB 191	
	PCB 26	PCB 93	PCB 165	PCB 200	
	PCB 25	PCB 95	PCB 146	PCB 170	
	PCB 31	PCB 91	PCB 188	PCB 190	
	PCB 50	PCB 56	PCB 153	PCB 199	
	PCB 28	PCB 60	PCB 132	PCB 196	
	PCB 20	PCB 92	PCB 105	PCB 203	
	PCB 33	PCB 84	PCB 141	PCB 189	
	PCB 53	PCB 90	PCB 179	PCB 208	
	PCB 51	PCB 101	PCB 137	PCB 195	
	PCB 22	PCB 99	PCB 176	PCB 207	
	PCB 45	PCB 119	PCB 130	PCB 194	
	PCB 46	PCB 83	PCB 138	PCB 205	
	PCB 69	PCB 97	PCB 163	PCB 206	
	PCB 52	PCB 117	PCB 164		

Table 6. Summary of Data Collected

Site Type	Gas Type	Number of Samples from former GTI projects	Number of Samples from industrial customers	Number of Samples from literature search	Total number of samples
Dairy Farm	Biogas	12	42	5	59
Dairy Farm	Biogas (Partially Clean)	8	-	-	8
Dairy Farm	Biomethane	23	-	-	23
Landfill	Biogas	14	166	46	226
Landfill	Biomethane	16	-		16
Wastewater Treatment Plant	Biogas	5	133	20	158
Wastewater Treatment Plant	Biomethane	5	-	-	5
Natural Gas	Natural Gas	40	24	-	64
Total					***559***

1.1.3 *Task 2 – Microbial/Chemical Profile in Raw Biogas Pipeline*

Twelve raw biogas samples from 10 dairy farms in the mid-western, eastern and western regions of the U.S. were collected in the previous Dairy Farm Biogas project for determination of major raw biogas components and corrosion-related bacteria population carried over from anaerobic digestion processes. In addition, a condensate sample was collected in this project for determination of microbial and chemical profiles in raw biogas pipelines. The data will be used to formulate a synthetic condensate and bacteria consortium for Task 3 experiments to determine the corrosion effect of microbes on metal pipelines and collect data for MIC modeling in Task 4.

1.1.2.1. Major gas components in raw biogas

All twelve raw biogas samples were analyzed for major components such as methane, carbon dioxide, oxygen, and a handful of other compounds. This analysis was performed using ASTM D1946. Table 7 includes only the compounds that had observed concentrations above the method detection limit. For example, of the 12 raw biogas samples tested, only 11 raw biogas samples contained hydrogen sulfide above the method detection limit with an average concentration of 0.31% ± 0.15%.

Table 7. Results from Major Components Analysis for 12 Raw Biogas Samples

Compound	Detection Limit (Mol%)	Samples Above Detection Limit	Average (Mol%)	Standard Deviation	Min (Mol%)	Max (Mol%)
Carbon Dioxide	0.03	12	35.5	4.17	28.57	40.39
Oxygen/Argon	0.03	12	0.74	0.82	0.22	2.94
Nitrogen	0.03	12	3.08	3.46	0.64	12.67
Methane	0.002	12	60.42	5.40	49.03	68.58
Hexane Plus	0.0001	7	0.0002	0.0001	0.0001	0.0004
Ammonia	0.001	1	0.004	NA	0.004	0.004
Hydrogen Sulfide	0.000005	11	0.3085	0.1473	0.148	0.6570
Carbonyl Sulfide	0.000005	12	0.000154	0.0001	0.000034	0.000409

As indicated in Table 7, hydrogen sulfide is a significant component in raw biogas and a thorough speciation analysis using ASTM D6228 was performed on the 12 raw biogas samples to identify the species of sulfur compounds as shown in Table 8. The raw biogas samples tested had an average total sulfur concentration of 2830 ppmv (168 grains/100 scf) with a range from 0.34 ppmv (0.02 grains/100scf) to 6580 ppmv (390 grains/100 scf). While the major sulfur species is hydrogen sulfide for almost all of samples analyzed, most of samples also contain other sulfur compounds in various quantities such as sulfur dioxide, carbonyl sulfide, mercaptan, etc. It should be noted that one raw biogas sample collected from one of the dairy farms contained an unusually low amount of total sulfur, 0.02 grains/100scf. No explanation for this low concentration has been confirmed but the weather conditions on that day were very

unfavorable for sample collection. The recorded ambient temperature during this specific sampling event was -8°C, therefore it is possible that the integrity of the sample was compromised. If this sample is removed from the raw biogas data set, then the new calculated average for total sulfur concentration would be 182 grains/100 scf. Unlike the average total sulfur concentration observed from the raw biogas samples, the total sulfur concentration typically found in pipeline tariffs are much lower and range from 0.5-20 grains/100 scf (AGA 2008).

Table 8. Results from Sulfur Analysis for 12 Raw Biogas Samples.

Compound	Detection Limit (ppmv)	Samples Above the Detection Limit	Average (ppmv)	Standard Deviation	Min (ppmv)	Max (ppmv)
Hydrogen Sulfide	0.05	11	3090	1470	1480	6570
Sulfur Dioxide	0.05	10	1.31	2.36	0.07	7.73
Carbonyl Sulfide	0.05	12	1.54	1.05	0.34	4.09
Carbon Disulfide	0.05	3	0.09	0.072	0.03	0.17
Methyl Mercaptan	0.05	11	2.00	2.01	0.25	6.12
Ethyl Mercaptan	0.05	11	0.20	0.072	0.07	0.30
i-Propyl Mercaptan	0.05	11	0.55	0.39	0.09	1.35
n-Propyl Mercaptan	0.05	4	0.08	0.012	0.06	0.09
t-Butyl Mercaptan	0.05	4	0.27	0.242	0.05	0.60
Dimethyl Sulfide	0.05	9	0.30	0.321	0.09	0.32
Dimethyl Disulfide	0.05	1	0.32	NA	0.32	0.32
Diethyl Disulfide	0.05	1	0.15	NA	0.15	0.15
Thiophene	0.05	7	0.15	0.068	0.25	0.26
Total Sulfur (ppm)	NA	12	2830	1670	0.34	6580
Total Sulfur (As Grains/100 SCF @ 14.73 psia, 60°F)	NA	12	168	98	0.02	390

1.1.2.2. Major microbial components in raw biogas

Microbiological analyses were performed in 10 raw biogas samples to determine 1) the number of total (live and dead) heterotrophic bacteria and various corrosion causing bacteria (APB, IOB, and SRB), 2) the number and identity of live bacteria, and 3) the number and identity of bacterial spores. The number of total bacteria and total corrosion-causing bacteria including both dead and live bacteria on the filter was determined by a genetic method (qPCR) by targeting specific genes present in the target microorganisms, and the data was reported as numbers per 100 scf of gas sample. The number of live bacteria and spore was determined by inoculating samples (phosphate buffer saline suspension of filter) to appropriate bacteria medium and incubated at 37 ºC for a pre-determined time, and the data was reported as colony-forming unit (CFU) per 100 scf of gas sample.

The filter sample was placed in a 50-ml tube with 30 ml of sterile phosphate buffered saline (PBS, pH 7.2 ± 0.1), voterxed for 5-10 sec, and sonicated for 2 min ± 5 sec in waterbath sonicator filled with fresh aqueous solution of 0.3% vol/vol Tween 80. After sonication, the filter suspension was used for Most Probable Number (MPN) test, Spore Enumeration, and DNA extraction. The MPN test determines the number of live heterotrophic bacteria in the filter samples carried over from anaerobic digestion process. MPN tests were performed in thioglycolate medium (TG media) in triplicate with serial dilutions of filter suspension samples. After 7 days incubation at 37 ºC aerobically and anaerobically, the positive culture bottles were scored and the number of heterotrophic bacteria determined using a statistically derived table (Most Probable Number from Serial Dilution, Bacteriological Analytical Manual, FDA, February 2006). The positive MPN culture then was used for DNA extraction. A second part of the filter suspension sample was used for Spore Enumeration using a Pour Plate Procedure modified from NASA standard assay NHB 5340.1D. The sample suspension is heat-shocked at 80 ± 2 ºC water bath for 15 min to kill vegetative cells, inoculated onto Tryptic Soy Agar (TSA) medium, incubated at 37 ºC aerobically and anaerobically for 3 days before colony counts to determine the number of live spores in the filter sample. The spore colonies were used for DNA extraction.

DNA extraction for filter suspension samples without prior growth and positive MPN culture in TG media and spore colonies on TSA plate after growth was performed using FastDNA SPIN Kit for Soil (MP Biomedicals LLC). DNA from filter suspension samples was used for determination of bacterial identity and qPCR quantification of total bacteria, total APB, total SRB, and total IOB. DNA from MPN culture and spore colonies was used for determination of identities of live bacteria and spores. qPCR quantification of specific groups of bacteria was achieved by targeting bacterial 16S rRNA, *ackA* and *buk*, *dsrAB*, and IOB 16S rRNA genes, respectively, following the instructions of the Rotor-Gene 3000 4 Channel Multiplexing System and QuantiTect PCR kits (Qiagen Inc., Valencia, California).

The extracted DNA was amplified with polymerase chain reaction (PCR) using various primers specific to the target bacteria groups and the PCR products were used for determination of bacteria identities in the samples. For heterotrophic bacteria, universal primer pair BA8F/UN1492R was used to target 16S rRNA gene, and if it failed due to low quantity of target DNA in the samples, a second universal primer pair BA338F/BA1392R was used for a nested

PCR to amplify target 16S rRNA gene from the samples. For acid-producing bacteria (APB), two pairs of primers were used to amplify *ackA* (ackA-3F and ackA-4R) and *buk* (buk-5F and buk-6R) genes, respectively. Two pairs of primers (IOB-F486 and IOB-R1132, and Gall-F704 and IOB-R1000) were also used to amplify 16S rRNA gene from iron-oxidizing bacteria (IOB).

The PCR products were purified using QIAquick PCR Purification Kit, and the purified PCR products were inserted into pGEM-T Easy Vector System I (Promega Corp., Madison, Wisconsin). The vectors were then transformed into DH5α Subcloning Efficiency Chemically Competent Cells purchased from Invitrogen (Carlsbad, California), and the cells were inoculated onto LB agar medium for screening of white colonies after overnight incubation at 37 ºC. The white colonies were picked and their DNA prepared for sequencing. The sequences were analyzed with the Blast program in GenBank database and identities of heterotrophic bacteria, APB, and IOB were determined.

The results from genetic quantification (qPCR) on filter samples indicates how many heterotrophic bacteria are generated by anaerobic digestion and have remained in the raw biogas stream, whether or not they are dead or still alive. APB, IOB, and SRB are major corrosion-causing bacteria, and the genetic tests indicate if the anaerobic digestion process might pose a pipeline corrosion risk if the raw gas is not treated. However, many microbes may not be able to survive the adverse environment during anaerobic digestion, and the number of microbes which are still alive in the raw biogas stream is supposed to be much smaller. In addition, downstream clean-up processes may also kill some of live microbes carried over from the raw biogas. The number of live bacteria is more relevant to the risk the microbes pose to the integrity of pipelines. It is worth noting, though, that some bacteria may be killed during sampling process (filtration); as a result, the number of live bacteria retrieved from MPN test might be underestimated to a certain degree. Bacterial spores may survive very adverse environment such as clean-up process and sampling process, and may pose higher risks to pipeline integrity and human health.

The results in Table 9 indicated that most raw biogas samples carried an average of 2.72E+06 heterotrophic bacteria per 100 scf with a range of 5.81E+05 to 3.8E+07 per 100 scf from anaerobic digestion. In terms of more specific groups of bacteria, most raw biogas samples contained two major types of corrosion-causing bacteria - APB and IOB, with an average of 1.82E+04 and 2.52E+03 per 100 scf, respectively. SRB was detected only in 1 raw biogas sample, indicating that SRB are not a significant group of bacteria in anaerobic digestion process during biogas production.

The bacteria leaving the digester may be dead already due to the unfavorable environment in the digester, or may die after they were caught by a filter during the sampling process due to desiccation. Therefore, the live bacteria or spores detected by MPN test and Pour Plate method may only represent a portion of microbes which could survive the whole process, and might be more relevant to the pipeline integrity and health risk of consumers if the filter would fail for some reasons. Live aerobic bacteria were detected in all 10 raw biogas samples, and anaerobic bacteria in 8 samples, with a mean 4.04E+02 and 1.45E+02 per 100 scf, respectively. Only four of the 10 raw biogas samples tested positive for bacteria spores, containing an average number of 537 spores per 100 scf.

Table 9. Results from Biological Testing for 10 Raw Biogas Samples

Method	qPCR				MPN		Pour Plate
	Total Bacteria	Total APB	Total IOB	Total SRB	Live Aerobic Bacteria	Live Anaerobic Bacteria	Live Spores
	CFU/100 scf or #/100 scf						
Mean	2.72E+06	1.82E+04	2.52E+03	1.10E+02	4.04E+02	1.45E+02	5.37E+02
Standard Deviation	3.14	3.3	1.66	NA	2.75E+00	1.86E+00	1.74E+00
Minimum	5.81E+05	1.23E+03	1.02E+03	1.10E+02	9.82E+01	8.75E+01	2.48E+02
Maximum	3.80E+07	6.03E+04	5.09E+03	1.10E+02	2.11E+03	5.95E+02	8.51E+02
Samples above Detection Limit	10	9	8	1	10	8	4

1.1.2.3. Major microbial species in raw biogas

DNA samples collected from previous Dairy Farm Biogas project were used in this project to determine the microbial profile in raw biogas samples. There are four sources from which DNA was isolated: 1) directly from biogas filter, 2) from positive MPN culture incubated under aerobic condition, 3) from positive MPN culture incubated under anaerobic condition, and 4) from positive bacterial spore culture. The DNA from the above sources was used to determine the identities of heterotrophic bacteria, bacterial spores, and corrosion-related APB and IOB by targeting 16S rRNA genes or specific functional genes such as *ackA* and *buk* genes.

1.1.2.3.1. Identity of major heterotrophic bacteria

All 24 heterotrophic bacteria sequences isolated from three filter samples not grown in the culture medium were closely related to *Paenibacillus* sp. (Table 10). However, after the filter suspension samples were grown in culture medium aerobically, various *Bacillus* sp. were enriched and became the dominant heterotrophic bacteria (Table 11). They accounted for 58% of 24 sequences isolated, and majority of them were *B. licheniformis* (38%). When filter suspension samples were grown in culture medium anaerobically, all 16 sequences isolated were closely related to *Paenibacillus* sp., though different species from those directly from filter samples without growth (Table 12).

1.1.2.3.2. Identity of major bacterial spores

The identities of bacterial spores isolated from 4 positive culture samples were also determined (Table 13). Of 56 bacterial spore sequences retrieved, the majority of them were identified as *Bacillus licheniformis* (48%), and various other *Bacillus* species (43%). Due to the resistance of their endospores to environmental stress, as well as their long-term survival under adverse conditions, most aerobic spore-formers are ubiquitous and can be isolated from a wide variety of sources. Hence, the occurrence of spore-forming bacteria in a certain environment is not necessarily an indication of habitat. However, it is generally accepted that the primary habitat

of the aerobic endospore-forming bacilli is soil. Aerobic spore-formers are considered as "normal flora" of the soil. They become metabolically active when suitable substrates for their growth are available.

Table 10. The Closest Relatives of Heterotrophic Bacteria Sequences Isolated directly from 3 Filter Samples without Growth using Universal Primers Targeting 16S rRNA Gene

Closest relative in Genbank	Genbank accession No.	% Identity	Frequency
Paenibacillus glucanolyticus	AB073189	99	3
Paenibacillus glucanolyticus strain FR1_105	EU373524	99	1
Paenibacillus sp. isolate P14-7	AJ297712	96	1
Paenibacillus sp. JAM-FM32	AB526335	99-100	19

Table 11. The Closest Relatives of Heterotrophic Bacteria Sequences Isolated from 3 Positive Aerobic MPN Cultures using Universal Primers Targeting 16S rRNA Gene

Closest relative in Genbank	Genbank accession No.	% Identity	Frequency
Bacillus licheniformis isolate CCM28B	FN433039	100	1
Bacillus licheniformis strain CICC 10087	GQ375232	100	1
Bacillus licheniformis strain CICC 10181	GQ375235	100	2
Bacillus licheniformis strain NBST2	GU011947	99	1
Bacillus licheniformis strain nju-1411-1	FJ915147	99-100	3
Bacillus licheniformis strain YP1A	EF105377	100	1
Bacillus sp. strain R-30915	AM910273	99	3
Bacillus sp. FE-1	EU271855	99	1
Bacillus sphaericus strain 601	DQ350820	98	1
Bordetella avium 197N	AM167904	98	2
Sporosarcina ginsengisoli	AB245381	96-99	2
Sporosarcina luteola	AB473560	100	1
Uncultured bacterium clone 101-68	EF157238	98	3
Uncultured bacterium clone 2G4-89	EU160423	98	1
Uncultured bacterium clone B1	FJ868757	96	1

Table 12. The Closest Relatives of Heterotrophic Bacteria Sequences Isolated from 2 Positive Anaerobic MPN Cultures using Universal Primers Targeting 16S rRNA Gene

Closest relative in Genbank	Genbank accession No.	% Identity	Frequency
Paenibacillus barengoltzii strain THWCS9	GQ284356	98-99	2
Paenibacillus barengoltzii strain THWCSN47	GQ284370	98	1
Paenibacillus sp. strain HanTHS1	AM283040	98	2
Paenibacillus sp. 5T01	AM162346	99	4
Paenibacillus sp. enrichment culture clone 9	FJ930068	99-100	7

The results indicate that the dominant heterotrophic bacteria or bacterial spores in raw biogas derived from dairy biomass belong to two genera, i.e. *Paenibacillus* and *Bacillus*. *Bacillus* is large and diverse genus of bacteria in the Family Bacillaceae. *Paenibacillus* is a genus split off from genus *Bacillus* in 1997 based on ssRNA analysis. Both belong to Class *Bacilli* and Order *Bacillales*. They are Gram-positive aerobic or facultative endospore-forming bacteria.

Collectively, the aerobic spore-formers are versatile chemoheterotrophs capable of respiration of most all substrates derived from plant and animal sources, including cellulose, starch, pectin, proteins, agar, hydrocarbons, and others, although simple organic compounds such as sugars, amino acids, organic acids are preferred. In some cases, they also ferment carbohydrates in a mixed reaction that typically produces glycerol and butanediol. Endospore forming bacteria play a significant role in the biological cycles of carbon and nitrogen. The majority of these are mesophiles, with temperature optima between 30 ºC and 45 ºC, but some are thermophiles with optima as high as 65 ºC. They are found growing over a range of pH from 2 to 11. In the laboratory, under optimal conditions of growth, *Bacillus* species exhibit generation times of about 25 minutes.

Table 13. The Closest Relatives of Bacterial Spore Sequences Isolated from 4 Positive Spore Cultures using Universal Primers Targeting 16S rRNA Gene

Closest relative in Genbank	Genbank accession No.	% Identity	Frequency
Bacillus anthracis str. 'Ames Ancestor'	AE017334	100	1
Bacillus bataviensis strain MSU1210	AY647284	99	1
Bacillus licheniformis ATCC 14580	CP000002	99-100	7
Bacillus licheniformis strain B8	EU117278	97	1
Bacillus licheniformis strain CICC10094	AY842873	99	4
Bacillus licheniformis strain MML2501	EU344793	99-100	4
Bacillus licheniformis strain MZ-14	EU586786	100	1
Bacillus licheniformis strain TCCC11009	EU231623	99	2
Bacillus licheniformis strain YP1A	EF105377	99-100	2
Bacillus licheniformis strain YRL03	EU373408	99-100	6
Bacillus sp. BT97	DQ358737	97	1
Bacillus sp. By137(B)Ydz-ss	EU070408	100	1
Bacillus sp. CBD 118	DQ374636	99	1
Bacillus sp. CSS-4	DQ084465	99-100	3
Bacillus sp. DCA-5	DQ238044	100	2
Bacillus sp. MO15	AY553108	97	1
Bacillus sp. N6	AB043854	99-100	8
Bacillus thuringiensis serovar konkukian strain I	EU438936	100	3
Bacillus thuringiensis strain KR19-22	EU414475	100	1
Clostridium beijerinckii NCIMB 8052	CP000721	99-100	2
Clostridium puniceum	X71857	99	1
Paenibacillus sp. MB 2039	AY257871	99	1
Uncultured Bacillus sp. clone ACf137	AM489497	99	2

P. glucanolyticus is rod-shaped, motile, facultative anaerobic endospore-forming bacteria that hydrolyze various β-blucans, including carboxymethyl cellulose and pustulan (Alexander and Priest 1989). Cells of *P. glucanolyticus* are long (usually >3.0 µm) and thin (<0.9 µm), and produce oval terminal spores that markedly distend the sporangium. Colonies are flat, smooth, and opaque and are motile during growth on dry nutrient agar plates. *P. glucanolyticus* degrades cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, raffinose, salicin, sucrose, trehalose, and D-xylose, and produces acids.

B. licheniformis is Gram-positive, rod-shaped, motile, aerobic endospore-forming thermophilic bacteria (with a diameter < 0.9 μm) that hydrolyze sugars fermentatively. Colonies of *B. licheniformis* are round, surface smooth, flat, margin irregular and 2-4 mm in diameter. Ellipsoidal spores are produced in not swollen sporangia and placed centrally (Pridal 2001). *Bacillus licheniformis* has been associated with a range of clinical conditions, food spoilage and incidents of food-borne gastro-enteritis. *B. licheniformis* has also been associated with septicaemia, peritonitis, ophthalmitis, and food poisoning in humans, as well as with bovine toxaemia and abortions. Food-borne *B. licheniformis* outbreaks are predominantly associated with cooked meats and vegetables (http://en.wikipedia.org/wiki/Bacillus_licheniformis). *B. licheniformis* is a common contaminant of dairy products; it is the most common aerobic spore-forming bacteria isolated from dairy farm (Scheldeman, Pil et al. 2005). The optimal growth temperature is around 50°C, though it can survive at much higher temperatures. Optimal temperature for enzyme secretion is 37°C. It can exist in spore form under harsh environments or in a vegetative state when conditions are good.

1.1.2.3.3. Identity of major acid-producing bacteria (APB)

Since the results from qPCR analysis (Table 9) indicated the widespread existence of two major types of corrosion-related bacteria (APB and IOB), the identities of dominant APB and IOB species were determined by targeting *ackA* and *buk* genes for APB, and 16S rRNA genes for IOB. The attempt to directly amplify *ackA* and *buk* genes from filter suspension samples without prior growth in the medium failed; therefore the dominant profile of APB was derived from samples after they were inoculated to, and grown in, the culture medium (Table 14 and Table 15). *Bacillus sp.* (e.g. B. licheniformis and B. cereus), *Geobacillus sp*., and *Clostridium sp*. were the dominant acid-producing species in raw biogas derived from dairy biomass.

Table 14. The Closest Relatives of APB Sequences Isolated from 3 Positive Aerobic MPN Cultures using Primers Targeting *ackA* and *buk* Genes

Closest relative in Genbank	Genbank accession No.	% Identity	Frequency
Bacillus licheniformis ATCC 14580	CP000002	93-100	34
Clostridium acetobutylicum ATCC 824	AE001437	74-75	2
Geobacillus sp. WCH70	CP001638	75-76	3
Geobacillus sp. Y412MC10	CP001793	76	1
Methanosarcina mazei strain Goe1	AE008384	73	1

Table 15. The Closest Relatives of APB Sequences Isolated from 2 Positive Anaerobic MPN Cultures using Primers Targeting *ackA* and *buk* Genes

Closest relative in Genbank	Genbank accession No.	% Identity	Frequency
Bacillus anthracis str. A0248	CP001598	79-82	9
Bacillus cereus E33L	CP000001	98-100	7
Bacillus pumilus SAFR-032	CP000813	90-92	3
Clostridium acetobutylicum ATCC 824	AE001437	73-77	5
Geobacillus sp. Y412MC10	CP001793	74-75	8
Methanosarcina acetivorans str. C2A	AE010299	75	1
Vibrio fischeri MJ11 chromosome I	CP001139	74	1

1.1.2.3.4. Identity of major iron-oxidizing bacteria (IOB)

The IOB sequences derived from filter suspension samples were very diverse (Table 16). The majority of sequences (18 out of 27) isolated were closely related to sequences of bacteria which have not been cultured successfully. Four sequences were closely related to *Acidovorax* species. A well-known IOB *Gallionella ferruginea* was only detected once. After filter suspension samples were grown under aerobic or anaerobic conditions, *Bacillus* and *Paenibacillus* were found to be the dominant species (11 out of 25 and 35 sequences, respectively) (Table 17 and Table 18). Other well-known IOB *Sphaerotilus* and *Gallionella* were only detected once, respectively. Fifteen sequences (out of 35) isolated after anaerobic growth were closely related to uncultured bacterial sequences. *Gallionella*, *Leptothrix*, and *Sphaerotilus* are the three major genera of iron-oxidizing bacteria. Only a few species within these genera have been isolated from the environment and successfully cultured in the laboratory. The primers designed based on limited number of sequences deposited in Genbank database might not be optimal, and may amplify some non-target DNA sequences from other bacteria. The identity results appeared to confirm this assumption since many *Bacillus* and *Paenibacillus* sequences had been isolated from the samples. In summary, IOB might not be a significant corrosion-related population in raw biogas samples.

Table 16. The Closest Relatives of IOB Sequences Isolated directly from 2 Filter Samples without Growth using Primers Targeting IOB 16S rRNA Gene

Closest relative in Genbank	Genbank accession No.	% Identity	Frequency
Acidovorax facilis strain TSWCSN46	GQ284412	100	1
Acidovorax sp. PPs-5	FJ605421	99	2
Acidovorax temperans strain 2R3-13	GU169008	100	1
Beta proteobacterium ASRB1	AY612302	99	1
Gallionella ferruginea	L07897	100	1
Paenibacillus barengoltzii strain THWCSN14	GQ284362	99	1
Paenibacillus sp. 5M01	AM162347	99	1
Paenibacillus sp. D273a	FJ430033	100	1
Uncultured bacterium clone MRA3016	FN428762	99-100	2
Uncultured bacterium clone nbw217a04c1	GQ074849	100	1
Uncultured bacterium clone nbw335c11c1	GQ090536	100	2
Uncultured bacterium clone nbw390c06c1	GQ096609	99	1
Uncultured bacterium clone nbw403b10c1	GQ098239	99	1
Uncultured bacterium clone nbw518g07c1	GQ104363	100	1
Uncultured bacterium clone nbw520b12c1	GQ104478	99	1
Uncultured bacterium clone nbw530e12c1	GQ105704	100	1
Uncultured bacterium clone nbw534f08c1	GQ106415	100	1
Uncultured bacterium clone nbw579c08c1	GQ106258	100	1
Uncultured bacterium clone nbw638f09c1	GQ114515	100	1
Uncultured bacterium clone nbw639h01c1	GQ114609	100	1
Uncultured bacterium clone nbw680e07c1	GQ114021	100	1
Uncultured bacterium clone nbw906h06c1	GQ032518	99	1
Uncultured beta proteobacterium clone R64LS	FM863753	100	1
Uncultured Ralstonia sp. clone 1P-1-G07	EU704794	99	1

Table 17. The Closest Relatives of IOB Sequences Isolated from 3 Positive Aerobic MPN Cultures using Primers Targeting IOB 16S rRNA Gene

Closest relative in Genbank	Genbank accession No.	% Identity	Frequency
Bacillus boroniphilus strain PL69	GU001903	99	1
Bacillus licheniformis strain 3EC7A1	EU304968	99	1
Bacillus licheniformis strain ES_MS4c	EU888508	99	1
Bacillus sp. BCL23-1	EF026994	100	1
Bacillus sp. HB1	FM208185	99	1
Bacillus sp. JJM-1	GU132507	99-100	5
Bacillus sp. MB66	AB518978	99	1
Pigmentiphaga sp. Zn-d-2	EU170477	98-99	9
Ralstonia sp. RS2	AB503703	100	1
Salmonella enterica strain st8r	FJ544366	100	1
Sphaerotilus sp. HS	EU636006	99	1
Uncultured Bacillus sp. clone QNSW24	FJ384500	99	1
Uncultured bacterium clone NCH1312/73f	EU560864	96	1

Table 18. The Closest Relatives of IOB Sequences Isolated from 2 Positive Anaerobic MPN Cultures using Primers Targeting IOB 16S rRNA Gene

Closest relative in Genbank	Genbank accession No.	% Identity	Frequency
Bacillus sp. JJM-1	GU132507	99	1
Gallionella ferruginea	L07897	99-100	5
Paenibacillus barengoltzii strain SAFN-125	DQ124699	99	1
Paenibacillus barengoltzii strain THWCSN13	GQ284361	99	3
Paenibacillus barengoltzii strain THWCSN14	GQ284362	99	2
Paenibacillus sp. 5M01	AM162347	99	1
Paenibacillus sp. AT5	GU097198	98	2
Paenibacillus sp. D273a	FJ430033	100	1
Paenibacillus sp. oral taxon 786 strain F0064	GQ422747	99	1
Pseudoxanthomonas taiwanensis strain NFC7-12	EU250946	99-100	3
Uncultured bacterium clone AR18	EU008373	99	2
Uncultured bacterium clone EU32	EU008374	99	1
Uncultured bacterium clone EU40	EU008371	99	1
Uncultured bacterium clone EU40A	EU008377	99	1
Uncultured bacterium clone nbw335c11c1	GQ090536	100	5
Uncultured bacterium clone nbw520b12c1	GQ104478	100	2
Uncultured bacterium clone nbw641c05c1	GQ115240	100	1
Uncultured bacterium clone p02_E05	FJ602432	100	1
Uncultured bacterium clone TSBAR002_E10	AB486284	99	1

Note: Analysis of condensate sample is in progress. The results will be reported in the next quarterly report.

1.1.4 *Task 3 - Lab Evaluation of Microbial Corrosion under Simulated Field Conditions*

1.1.3.1. Major Modeling Parameters

Corrosion is mainly the consequence of electrochemical reactions, influenced by the physical/ chemical environment at the metal surface, such as oxygen, salts, pH, redox potential, and conductivity, etc. MIC is electrochemical corrosion influenced by the presence or activities of microorganisms. Microorganisms growing at the metal surface form a biofilm and release chemicals or electrochemically active minerals, which alter the rates and types of electrochemical reactions at the biofilm-metal interface and result in various types of corrosions (e.g. pitting, crevice corrosion, under-deposit corrosion, and galvanic corrosion)

Biogas, generated through the anaerobic digestion from a variety of biomass sources, is one of the fastest growing renewable fuels. Within the past few years, there has been enthusiasm and investment in bioconversion of waste products into quality fuel, encouraged by political and public pressure to create and use "green" energy products. Local gas distribution companies (LDCs) are poised to take delivery of (interchange) cleaned biomethane into their existing lines for general distribution. However, based upon its source (dairy waste, landfill, wastewater sludge, agricultural waste, etc.), the raw biogas may contain constituents that may affect pipeline integrity and system operations, and possibly impede pipeline safety. One such known constituent is bacteria associated with microbiologically-induced corrosion (MIC) in the biogas produced and carried over from the anaerobic digestion process. However, the relationship between the numbers of specific MIC bacteria introduced into the pipe, internal pipe conditions, and severity of metallic pipeline corrosion has not been fully understood (Little, Lee et al. 2006; Zhu 2007) despite the fact that MIC has been long recognized as one of the major causes of corrosion of metal pipes (Graves 1996; Angell 1999; Batista 2000; Kholodenko 2000).

Raw biogas, saturated with moisture, contains hundreds of live bacteria (Table 9) including those known to cause MIC (e.g., APB, IOB, and SRB) from the anaerobic digestion process. The properties of condensate formed in gathering pipeline are affected by biogas composition (CO_2 and H_2S, etc) (Table 7), dissolved chemicals and nutrients from the anaerobic digestion process, which in turn, influence the dominant bacterial profile and microbial interactions with the metal surfaces. The potential impact of microbial corrosion on the integrity of metallic gathering pipelines must be addressed.

Consequences of the direct introduction of live microbes to metallic pipeline networks are unknown. A clear understanding of such potential integrity impact is crucial to safe introduction of biogas into metallic natural gas networks. In addition, a predictive tool to foretell MIC severity under field conditions is necessary for the effective management of pipeline integrity, especially for gathering lines containing the raw biogas.

Internal corrosion in raw biogas lines are affected by many factors or combination of factors including CO_2, H_2S, organic acid (mainly acetic acid), microbes, oxygen, chloride, etc. The focus on a single mechanism such as microbial corrosion is therefore not appropriate or practical in an actual pipeline system (Pots and Kapusta 2005). The development of the MIC model has to include other factors which may interact with microbial activities and their metabolites, and change electrochemical characteristics at the metal-biofilm interface. Parameters which affect microbial growth and activities will probably affect the onset of microbial corrosion (i.e. pitting),

corrosion rate and severity. The parameters which may be included in the MIC model are nutrients (sulfate, fatty acids, total dissolved solids, utilizable nitrogen), CO_2, H_2S, O_2, pH of condensate, salinity, alkalinity, dissolved iron, sulfide, chlorides, bicarbonates, ferrous and ferric iron, and temperature. The final parameters which will be includes in our preliminary MIC model will be determined based on the results from Task 1 literature review and Task 2 sample analyses.

The major bacterial populations in raw biogas and condensate samples collected from gathering lines have been determined in Task 2, and the results used to formulate a major corrosion-related bacteria consortium to evaluate the microbial corrosion of metallic pipelines. In addition, chemical compositions and properties of typical condensate in raw biogas gathering line will be thoroughly analyzed in Task 2. Therefore, the microbial corrosion evaluation will be performed in synthetic condensate to mimic the field conditions typically found in raw biogas gathering line.

1.1.3.1.1. Major microbial parameters

The accurate diagnosis of MIC requires combination of microbiological, chemical, and metallurgical analyses. The microbiological indicators include detection and quantification of various microorganisms on metal-liquid interface, especially corrosive bacteria in biofilms formed on metal surfaces.

qPCR assays on 10 raw biogas samples indicated that most of samples contained two types of corrosion-causing bacteria – APB and IOB (Table 9). However, after the samples were inoculated in TG media and incubated for 7 days at 37 ºC, qPCR on positive growth cultures indicated the presence of overwhelming number of APB in most of samples (data not shown). The identities of most sequences of heterotrophic bacteria or bacterial spores in raw biogas were closely related to the sequences of two bacteria genera, i.e. *Paenibacillus* and *Bacillus* (Table 10 to Table 13). Species determination of corrosion-related bacteria showed the presence of *Clostridium* and *Acidovorax* species, in addition to dominant *Paenibacillus* and *Bacillus* species (Table 14 to Table 18). IOB such as *Gallionella*, *Leptothrix*, and *Sphaerotilus* might not be a significant corrosion-related population in raw biogas samples. Therefore the bacteria consortium for modeling of MIC in raw biogas gathering line will consist of *B. licheniformis* ATCC 14580, *Paenibacillus barengoltzii*, *P. glucanolyticus*, and *C. acetobutylicum* ATCC 824.

1.1.3.1.2. Major chemical parameters

Water is required for microbial metabolism and growth and corrosion processes. Water quality parameters that are considered important to understanding internal corrosion and MIC for a particular industrial system include temperature, pH, alkalinity, sulfide, nitrite, dissolved gases (CO_2, H_2S, O_2, NH_3, etc.), total dissolved solid (TDS), chemical oxygen demand (COD), microorganisms (bacteria, algae, and fungi), etc. Raw biogas contains up to 40% CO_2 and 0.66% of H_2S (Table 7). Dissolved CO_2 and H_2S in water form carbonic acid and weakly acidic hydrogen sulfide which attack the metal. Dissolved oxygen might not be indicative as to the oxygen content within the biofilm. COD measures the concentration of electron donors available for sulfate or metal reduction; hence a low COD means a low risk of finding SRB and iron-reducing bacteria in the system. Chloride (Cl^-) ions are very aggressive and participate in many forms of corrosion, including MIC. Chloride ions from the electrolyte migrate to the anode to

neutralize any buildup of charge, forming heavy metal chlorides that are extremely corrosive to metal surface, particularly stainless steels.

Pope and Pope (Pope and Pope 1998) listed a series of chemical and metallurgical indicators for diagnosis of MIC in natural gas pipeline.

Chemical MIC indicators include:

1) Sulfide: a strong positive indication. SRB reduce sulfate to sulfide, which combines with Fe and forms FeS.
2) Sulfate: a positive indication that SRB-induced MIC may occur. $FeSO_4$ corrosion product is soluble but less aggressive than the chloride iron for steel corrosion.
3) Chlorides: a positive indication. Chlorides are known to breakdown protective oxide layers and are a cathodic depolarizer. Iron chloride is soluble and its formation promotes anodic dissolution of iron and steel.
4) Short-chain volatile fatty acids: a positive indication for growth of APB.
5) pH: a positive indication that MIC may occur at the site if pH is less than approx. 5.5.
6) Ferrous iron: a positive indication that MIC may have occurred at the site. This is especially true if iron sulfide(s) is present.
7) Ferric iron: a positive indication that corrosion may have occurred at the site. Can also be an indication that the sample was exposed to oxygen in the pipeline, or after the sample was collected. The information is of little value in distinguishing between MIC and other forms of corrosion.
8) Hardness: an indication that scaling can occur (if pH is above about 8.0 and carbonates are present) and, therefore, generalized corrosion may be less likely. However, this has little influence on the possibility that MIC may occur at the site.
9) Carbonate: a neutral indicator of the possibility of MIC but could be important in choices of mitigation measures. Carbonate can form scales which can prevent the successful application of inhibitors or biocides.

Metallurgical MIC indicators include:

1) Discrete deposits: a positive indication that MIC may occur at the site.
2) Deposit color: Black or gray deposit is a strong positive indication that MIC has occurred at the site. Black or gray deposits almost always suggest ferrous iron (a reduced form of iron often associated with MIC).
3) Under deposit pit: a strongly positive indication that MIC has occurred at the site
4) Shiny pit: a strongly positive indication of high acidity at the site and it indicates that MIC maybe active.
5) Larger pits composed of smaller pits: a positive indication that MIC has occurred at the site.

1.1.3.2. Instrumentation for Modeling Data Collection

One of the objectives in this project is to evaluate the severity of corrosion in raw biogas metallic lines under simulated field conditions posed by a consortium of dominant corrosion-causing bacteria from anaerobic digestion. The evaluation will be performed in a batch culture mode for short-term study and continuous culture mode for long-term assessment. Two

approaches will be used to assess the corrosion rates and collect data for the model construction: testing of metal coupons for determination of weight loss, corrosion rates and morphology, and, use of an electrochemical device (e.g., Multielectrode Array Sensors) to monitor the real-time corrosion rate and collect electrochemical data. Other parameters which will be monitored over time include changes in: 1) bacterial numbers and profiles, and, 2) the composition of the condensate.

Experiments for data collection of modeling parameters will be performed under simulated raw biogas gathering line conditions. The artificial condensate liquid will be synthesized based on the comprehensive analysis of the field condensate sample to mimic the chemical compositions, major corrosive bacteria species, pH, salinity, conductivity, redox potential, etc. In the experiments, the prevailing variables that control the microbial reactions and corrosion process such as concentration and composition of bacteria consortium, pH, temperature, and condensate composition and properties will be captured. The variation of these variables against time will be measured, and the results will be used to construct a preliminary mechanistic model to predict the rate of MIC posed by corrosion-causing bacteria in raw biogas metallic pipelines.

A simplified MIC process is shown in Figure 5 (Gu, Zhao et al. 2009). A corrosive species such as O_2, CO_2, H_2S, H+, Cl-, or SO_4^{2-} may pass between the bulk solution and the steel surface through an aqueous boundary layer and the two biofilm layers. When diffusion of the corrosive species in the solution boundary layer is much faster than in the biofilms, only the concentration and potential gradients in the biofilms require consideration for model construction. Such concentration and potential gradients can be affected by the chemical and electrochemical reactions and further by the microbial activities in the biofilms which may consume or produce the corrosive species. For instance, O_2, if present, could be completely depleted in the top film while conversion of SO_4^{2-} to H_2S by bacteria may occur in the SRB biofilm or at the steel surface.

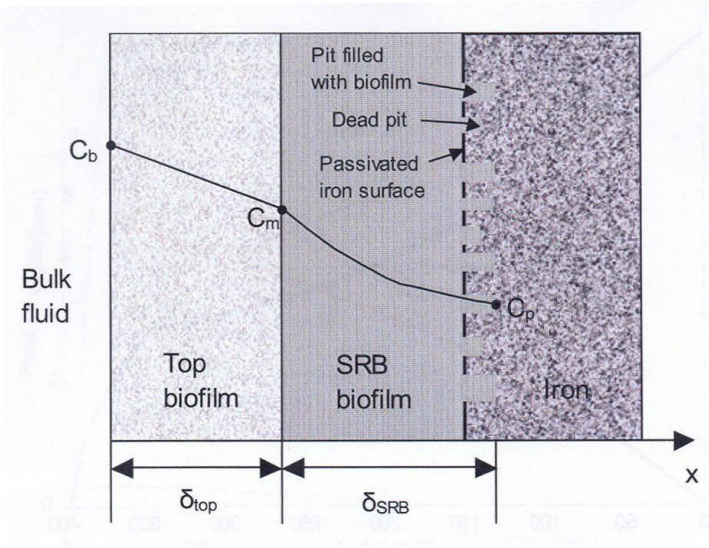

Figure 5: Illustration of a corrosive species passing from the bulk solution through a solution boundary layer (not shown) and two biofilm layers to the steel surface to result in MIC pits.

The basic equation to describe the transport process is the Nernst-Plank equation:

$$\frac{dc_j}{dt} - \nabla \cdot N_j + \sum R_k = 0 \quad (1)$$

coupled with the equation of electroneutrality:

$$\sum z_j c_j = 0 \quad (2)$$

where $N_j = (D_j \nabla c_j) + z_j u_j c_j \nabla \varphi + v c_j$ is flux and z_j, D_j, u_j and c_j are valence, diffusivity, mobility and concentration of the jth species, respectively. φ is electrostatic potential in solution. R_k is k^{th} irreversible reaction rate of the j_{th} species. v is velocity of the solution.

The above differential equations and the corresponding boundary conditions will be solved to determine the changes of the concentrations of each species and the rate of the pipe steel corrosion caused by corrosive species including microbial reactions (Song, Kirk et al. 2002; Song, Kirk et al. 2004; Song, Kirk et al. 2004; Song, Jones et al. 2005; Song and Sridhar 2006; Song 2008; Song 2008; Song and Sridhar 2008; Song and Sridhar 2008).

The setup below shows schematically how the effect of bacteria consortia on steel corrosion will be measured (Figure 6). The shaded labels 1, 2, 3, 4 are steel electrodes where 2, 3 are insulated from each other by epoxy as can be seen in their vertical projection on the right diagram. The electrodes are vertically inserted upward into a container containing sterile condensate solution. A membrane (shown in blue) extends upwards the epoxy insulator of small probes (1, 3, and 4), forming compartment above each electrodes. The membrane allows free passage of any solution species except microorganisms. Three different concentrations of bacteria consortia are injected into the membrane-surrounded compartment. The effect of activities of bacteria consortia on the corrosion rate of steel can be measured from current flows between small electrodes and the large electrode (2). The current is measured by a Keithley scanner which automatically records current flow between the electrodes at any given time interval. The counter and reference electrodes will be used to measure linear polarization resistance and thus corrosion rate at each electrode surface. A micro-pH electrode will be used to measure pH near the electrode surface. In separate cells, metal coupons will be used to measure the growth of bacteria consortia and the changes of chemical composition and properties in the condensate solution. The data collected will be used to develop a preliminary model for prediction of corrosion rate under influence of bacteria consortia.

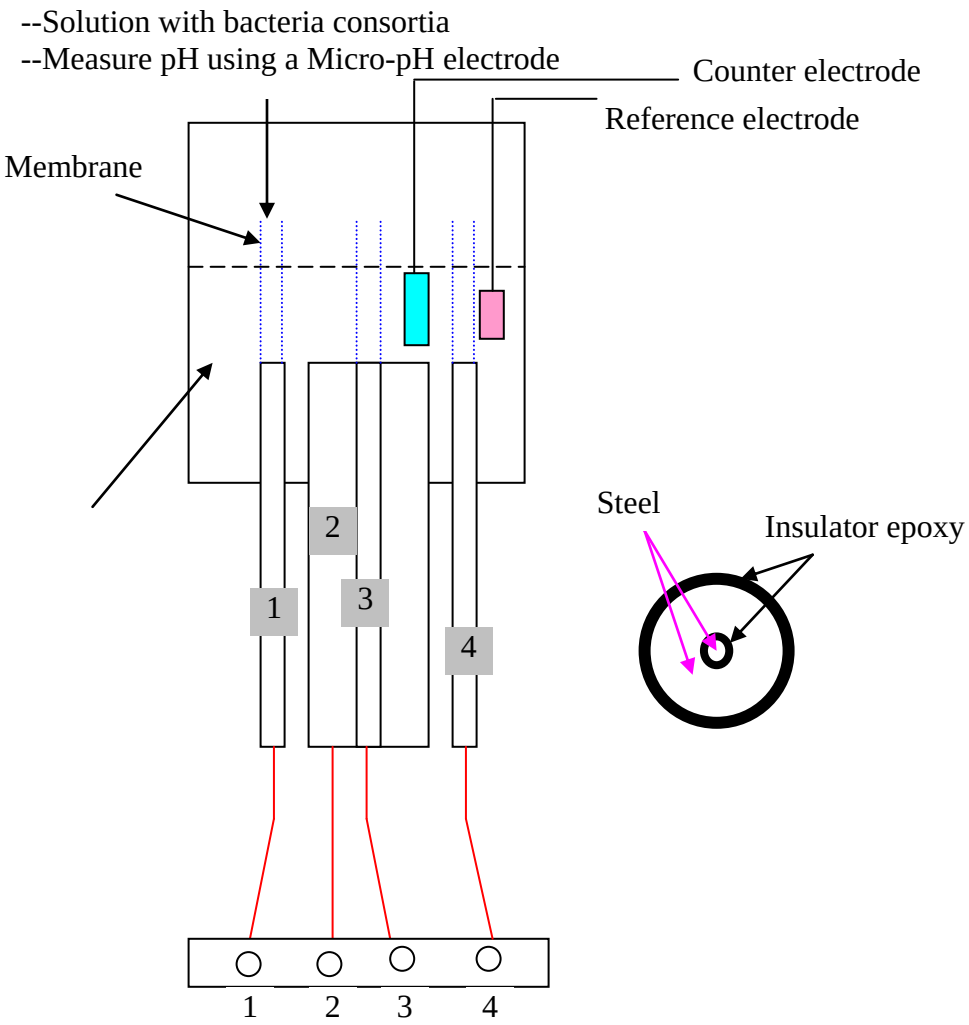

Figure 6. Schematics for electrochemical measurement of corrosion effect of bacteria consortium.

1.1.5 *Task 5 - Develop Compilation of Nonmetallic Materials*

A review of the nonmetallic materials that are currently used for building natural gas distribution systems were performed in this quarter. The overview of the plastic pipes, together with the physical and mechanical properties and the pipe joining methods are summarized in this report. A compilation of the elastomeric materials that are used as gasket, seal, etc for natural gas distribution systems are generated and the general description of each material including the physical, mechanical properties and chemical and environmental resistance are included. More detail analysis of each material will be performed in the next period to obtain a complete compilation of the material properties and their performance under the potential environment which may be encountered for biogas/biomethane applications.

1.1.1.1. Review of plastic pipes in gas distribution systems

Plastic pipes have been in continuous service in natural gas distribution system since 1960 with great success and over 90% of the pipe installed in US today is plastic (PPI 2006). According to ASME B31.8 and ASTM D2513, Polyethylene (PE), Poly Vinyl Chloride (PVC),

Polyamide (PA) and Crosslinked Polyethylene (PEX) are the thermoplastic used for gas pressure pipes. PE is the most used pipe material in natural gas distribution because of its outstanding corrosion, abrasion and crack propagation resistance, as well as its lightweight and flexibility to allow the pipe to be coiled or changed in direction with minimal use of fittings. It is estimated that nearly 95% of all new gas distribution pipe installations in North America that are 12" in diameter or smaller are PE pipes (PPI 2006).

According to ASTM D2513, PVC is only used to maintain or repair existing PVC gas piping and it is not the material for new construction of gas pipeline.

PA and PEX have a better temperature and chemical resistance than PE, but they are more expensive and have limited pipe size and manufacturers available, which limits their use to the applications that require superior temperature, pressure and chemical resistance.

Polyethylene Pipes

Besides the polyethylene polymer, PE piping material also contains colorants, stabilizers, anti-oxidants and other ingredients that enhance the properties. It can soften and melt when heated, and harden when cooled. It is a semi-crystalline polymer with very low glass transition temperature (Tg) which endows PE pipes great toughness, impact strength and resistance to rapid crack propagation. Density, molecular weight and molecular weight distribution are the three basic characteristics that greatly influence PE processing and the end-use properties.

PE resin are classified according to their density, see Table 19 (ASTM D3350). The mechanical strength, chemical and temperature resistance increases with density. High density polyethylene (HDPE) is stronger and it is able to make thinner wall pipes that are lighter and cheaper. Both medium density polyethylene (MDPE) and HDPE pipes are used to make gas distribution pipes, while HDPE pipes are more popular for higher pressure rating. PE2708 (Formerly PE2406) and PE3608 (Formerly PE3408) are the designation codes used in ASTM D2513 for MDPE and HDPE respectively.

PE 4710 is the latest advancement of Bi-Modal HDPE, which offers higher service pressure, reduced wall thickness and better overall mechanical properties. The resistance to slow crack growth (SCG) of PE4710 is considered to be a significant improvement over past HDPE pipes. This improved SCG resistance should make the pipe integrity less affected by the scrapes or small gouges incurred during installation.

In addition to natural gas gathering and distribution, HDPE pipe has been used for landfill and sewer application because it is resistant to hydrogen sulfide, low concentration sulfuric acid, bacteria and some other chemicals that present in sewage and cause severe corrosion of metal pipes (PPI 2009).

Crosslinked Polyethylene

PEX is formed by chemical joining polyethylene molecules to form a thermoset material in order to increasing the material's application temperature, and improve its resistance to environmental stress cracking, slow cracking and chemical degradation.

PE can be crosslinked by Peroxide, Vinylsilane or Beta irradiation, and the pipe properties are slightly different depending on the crosslinking method. Because of the crosslinking, PEX does not melt when heated and it can not be joined by heat fusion.

In general, the typical maximum service temperature for thermoplastic PE pipe is 140 ºF, while the service temperature for PEX can be raised to at least 212 ºF and sometimes as high as 248 ºF (PPI 2008). The typical physical, mechanical and thermal properties for MDPE, HDPE and PEX are listed in Table 20. (AGA 2006).

Polyamide Pipes

Polyamide, which is generally known as Nylon, is a semi-crystalline thermoplastic material. It is produced by condensation reaction of an amino group and a carboxylic acid with water eliminated. Generally, the number of the carbon atoms in the molecular chain determines the melting point and water absorption of this polymer. The higher the number of the carbon atoms are, the lower the melting point and water absorption. To improve the material properties, fillers, plasticizers, stabilizers and/or pigments can be added into the polyamide resin.

PA11 and PA12 are two resins in the polyamide family that have been investigated as an alternative material for natural gas distribution pipeline to replace steel pipes at higher pressure rating (Scholten 2008, Mason 2006, GRI 1999). The 11 and 12 represent the number of the carbon atoms in the repeat unit in polyamide molecular chain, see Figure 7. They are very similar from their molecular structure, but slightly different on physical and mechanical properties. They offer good mechanical properties with a combination of strength, toughness and hardness over a wide range of temperature. The continuous operating temperature of PA is -40 to 100 ºC.

In addition to the most of the benefits PE offers, PA 11 and PA12 also provide better chemical and temperature resistance as well as the higher mechanical strength. For this reason, PA11 and PA12 are very likely to be used as plastic piping in natural gas distribution system at pressure higher than 100 psi where PE is not suitable. Various demo-projects have been taken in US to verify the use of polyamides pipes at pressure above 100 psi.

1.1.1.2. Review of thermoplastic pipe jointing methods

There are three common methods for joining thermoplastic pipe: heat fusion welding, electrofusion and mechanical fitting. Though all the three methods provide leak free piping system in the gas distribution system, heat fusion and electrofusion eliminate the need of using the type of fittings that require elastomeric gasket materials and prevent the potential leaking problem due to the aging of elastomers overtime.

Heat Fusion Welding

Heat fusion welding is the most common method of joining thermoplastic pipe. A heater plate is inserted between the ends of two pipes to heat up the pipe ends to the melting point. The heater plate is then quickly removed and the melted pipe ends are drawn together with a specified force hold the ends together during the cool down. The formed joint is as strong as the pipe itself and leak-free.

Electrofusion

Electrofusion is used for both joining pipes and pipes to fittings. Heating elements are pre-installed in the couplers or fittings that are used to join the pipes. By applying electricity to the surrounding heating elements, the pipe is heated up, expand and fill the gap between pipe and fitting. Once the melting temperature is reached, the two pipe surface start to melt and weld together under clamping pressure. The melt from the couplers or fittings combines with that from

the pipe and bond the pipes to the couplers or fittings. When the current is terminated, the joint slowly cools under clamping pressure and form a high-tensile, leak-tight joint.

Mechanical fittings

Mechanical fittings are only used to connect thermoplastic pipe to other piping, valves, meters, etc. Because of forming crosslink in PEX, PEX pipe can't be heat fusion joined. It is possible to join PEX pipe using non-crosslinked PE electrofusion fittings, but the performance of such joints has not been fully investigated for all types of PEX piping (AGA handbook). Mechanical fittings are required to join PEX piping in fuel gas application.

1.1.1.3. Review of elastomers in natural gas distribution systems

Elastomers have been used as mechanical coupling seals and gaskets, meter and regulator diaphragms, boots, O-rings, flange seals, and valve seats, etc. They are amorphous polymers existing above their glass transition temperature, and are soft and flexible at room temperature. In addition to the base elastomeric polymer material, most of the elastomers also contain many additives including reinforcing agents, fillers, plasticizers, curing agents, accelerators, promoters, antioxidants and pigments. The variation of the formulations creates different type of elastomers with various physical, mechanical and chemical properties which are suitable for different type of applications and environment. Table 21 list the type of elastomers that are mostly used in natural gas distribution systems and below are a brief summary of the basic properties of these elastomers.

Butadiene-Styrene (SBR)

SBR is a synthetic rubber copolymer consisting of styrene and butadiene. It has very similar physical and mechanical properties as natural rubber. It is used as gasket material in natural gas distribution system, and the operating temperature is between -40 and 212 ºF. It is resistant to water, dilute acids, alkalies and alcohols but sensitive to oil, gasoline, hydrocarbons and oxidizing agents.

Butadiene-Acrylonitrile (NBR)

NBR is a family of unsaturated copolymers of 2-propenenitrile and various butadiene monomers (1,2-butadiene and 1,3-butadiene). It has been used as gasket, O-ring, diaphragms, flange and quad seals in natural gas distribution systems. The physical and mechanical properties of NBR are very similar to natural rubber, but it has exceptional strength and elasticity at low temperatures. It can withstand a temperature range from -40 to 250 ºF, but the hardness is low at high temperature. It is generally resistant to oil, fuel, solvents, water and hydraulic fluid, but it swells slightly in aliphatic hydrocabons, fatty acids, alcohols and glycols. The chemical resistance of NBR can be improved by the increase of nitrile content in the material, but the flexibility of the material will be negatively impacted.

Polychloroprene (CR)

CR is a family of synthetic rubbers that are produced by polymerization of chloroprene. It is relatively impermeable to gases, and used as gasket and O-rings in natural gas distribution systems. It is resistant to indoor/outdoor aging and weathering, and doesn't soften or melt when heated. It also has a good low temperature resistance down to approximately 0 ºF without significant change of performance. Except for nitric acid, concentrated sulfuric acid and some

organic solvents, CR has outstanding resistance to water and a wide range of chemicals including dilute mineral acids, inorganic salt solutions, alkalies, oils and aliphatic hydrocarbons. It is also a good candidate for buried underground environment as bacteria, soil chemicals and wastes have little effect on it.

Ethylene-Propylene (EPDM and EPT)

EPDM and EPT are the synthetic hydrocarbon-based rubbers made from ethylene-propylene diene monomer and ethylene-propylene terpolymer respectively. They are used as seals, gaskets, diaphragms, and O-rings in natural gas distribution systems. These materials exhibit the excellent static and dynamic creep resistance, as well as the good impact, tearing, abrasion and cut growth resistance. The service temperature in air ranges from -65 to 250 ºF. EPDM/EPT can resist the attack from many solvents and chemicals, such as acetone, methyl ethyl ketone, ethyl acetate, weak acids, alkalies, detergents, etc., but can't resist hydrocarbon based solvents, oils or chlorinated hydrocarbons. They also have excellent resistance to hot water and steam, ozone, oxygen and weathering.

Polyamide Elastomers (PA11 and PA12)

PA11 and PA12 can be formulated to thermoplastic elastomer (TPE) by incorporating polyether segments into polyamide segments forming block copolymer. The elastic performance is improved by the polyether segments and becomes more apparent with the increase of polyether content in the copolymer. As a result, the copolymer exhibits a combination of high tensile strength, ductility and toughness, together with high impact strength and good elastic memory and abrasion resistance. PA11/PA12 elastomers resist sun light, weathering and ozone and various chemicals including alkalies, inorganic salts, hydrocarbons, fuels and organic acids, but have limited resistance to hydrochloric acid, sulfonic acid, or phosphoric acid. Some gaskets and diaphragms in natural gas distribution system are made of PA11 or PA12 elastomers.

Silicone and Fluorosilicone (SI and FSI)

Silicone rubbers have a Si-O-Si backbone rather than C-C backbone. Compare to the other C-C bonds elastomers, silicone rubbers are more stable and have good resistance to heat, ozone, UV and other ageing factors. A special characteristic of silicone rubbers is the extreme low glass transition temperature at about -197 ºF, which make these elastomers maintain elasticity at low temperature. Silicon rubbers are able to be operated at extreme temperature from -67 to 572 ºF without significantly lose their useful properties. The tensile strength, abrasion and tear resistance of silicon rubbers are relatively low compared to the other C-C backbone elastomers. They are chemically inert to many chemicals such as dilute acids, akalies, alcohols, oils and aliphatic hydrocarbons and have excellent water resistance, but they are excessive swelling in aromatic and chlorinated solvent.

Fluorosilicone is fluorovinylmethyl silicone rubber. It offers good resistance to solvents, fuel and oil which come from fluorocarbons together with high and low temperature stability which come from silicones. It is good as static seal but not qualified for dynamic use due to the limited strength, poor abrasion resistance and high friction tendencies.

Silicone and Fluorosilicone rubbers are used as gasket material in natural gas distribution system.

Fluoroelastomers (FKM)

FKM are fluorine-containing hydrocarbon polymers, they have good tensile strength especially at high temperatures and excellent resilience. They are relatively impermeable to air and gases, and have exceptional resistance to oils and chemicals at elevated temperatures. They are used to make O-rings in natural gas distribution systems.

Perfluoroelastomers (FPM)

FPM combines the elastomeric properties of FKM and the chemical resistance of Polytetrafluoroethylene (PTFE) which is inert to over 1,600 chemicals, solvents and plasmas. They are also immune to sun, weathering and ozone and have better resistantance to swelling, embrittlement, and aging than any other elastomer. They are thermal stable up to 600 ºF and have similar physical properties to FKM.

Table 19 Polyethylene Resin Type and Density

Type	Density (g/cm^3)
I	0.91-0.925 (low)
II	0.926-0.940 (medium)
III	0.941-0.959 (high)
IV	0.960 and above (high, homopolymer)

Table 20 Typical Properties of PE and PEX Pipes

Physical Properties	PE2708 (PE 2406)	PE3608 (PE3408)	PE4710	PEX
Density (g/cm^3) ASTM D792	**0.926-0.940**	**0.941-0.947**	**0.947-0.955**	**0.941-0.955**
Tensile Strength (Yield, 10^3 psi) ASTM D638	**2.6**	**3.2-3.5**	**3.3-3.8**	**3.5-4.0**
Modulus of Elasticity (10^5 psi) ASTM D638	**0.90**	**1.1**	**1.3**	**1.5**
Compressive Strength (10^3 psi) ASTM D695	**2.6**	**3.2**	**3.4**	**3.6**
Flexural Strength (10^3 psi) ASTM D790	**100**	**110**	**150**	**180**
Long Term Strength (10^3 psi) ASTM D1598 ASTM D 2837	**1.2-1.5**	**1.5-1.7**	**1.5-1.8**	**1.5-1.8**
Izod Impact Ft-lbs/in of notch (f) ASTM D256	**No break**	**3-24**	**6-24**	**6-24**
Heat Deflect Under Load (ºF at 264 psi) ASTM D 648	**140 at 66 psi**	**150-175**	**160-180**	**180-200**
Water Absorption (% in 24h) ASTM D570	**0.1**	**0.1**	**0.1**	**0.1**
Coefficient of Expansion (in/in/ºF×10^{-5}) ASTM D696	**7-12**	**7-12**	**7-12**	**7-12**
Methane Permeability (Ft^3.Mil/Ft^3/Atm/Day)	**4.2×10^{-3}**	**2.4×10^{-3}**	**2.4×10^{-3}**	**2.4×10^{-3}**

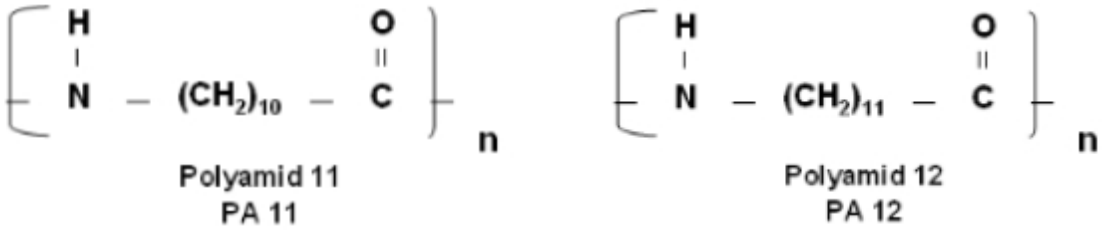

Figure 7. Structure of PA11 and PA12 molecular chain

Table 21 Elastomers in Natural Gas Systems

	Material Name	Other Names
1	Butadiene-Styrene	Buna-S; GR-S
2	Butadiene-Acrylonitrile	Buna-N; Nitrile; Perbunan; Nytek
3	Polychloroprene	Neoprene; Bayprene; Chloroprene
4	Ethylene-Propylene	Nordel; Royalene; Dutral
5	Polyamide (11 and 12)	Rilsan; Vydyne; Plaskin; Nylon
6	Silicone and Fluorosilicone	Polysiloxanes; Cohrlastic; Green-Sil; Parshiled; Baysilone; Blue-Sil
7	Fluoroelastomer	Viton; Fluorel; Technoflon
8	Perfluoroelastomer	Kalrez; Chemraz; Kel-F

Plans for Future Activity

Task 1 – Complete Task 1 in next quarter.

Task 2 – Complete Task 2 in next quarter.

Task 3 – Continue work activities

Task 4 – Initiate task

Task 5 - Continue work activities

Task 6 - Start work on Literature Search (Gap Analysis) for Material Compatibility Data

References

AGA (2008). American Gas Association. Transmission Measurement Committee. AGA Report No. 4A, Natural Gas Contract Measurement and Quality Clauses – 2008 DRAFT. Washington, DC: American Gas Association, 2008.

AGA, Plastic Pipe Manual for Gas Service, 8th edition, Washington DC: American Gas Association (AGA), 2006, pp. 1-30.

Alexander, B. and F. G. Priest (1989). "Bacillus glucanolyticus, a new species that degrades a variety of beta-glucans." Int. J. Syst. Bacteriol. 39: 112-115.

Allen, M. R., Braithwaite, A., & Hills, C. C. (1997). Trace Organinc Compounds in Landfill Gas at Seven U.K. Waste Disposal Sites. Environmental Science and Technology , 1054-1061.

An Engineering guide to Elastomer and Manufacturing Standards, Bloomfield: Minor Rubber Company Inc., 2001, pp. 1-30.

Angeles-Ch, C., et al. (2002). "Microbiologically Influenced Corrosion by Citrobacter in Sour Gas Pipelines." Materials Performance 41(8): 50-55.

Angell, P. (1999). "Understanding microbially influenced corrosion as biofilm-mediated changes in surface chemistry." Curr. Opin. Biotechnol. 10(3): 269-72.

Angostini, R. A. and R. D. Young (1990). "A case history: Investigations of microbially influenced corrosion in a west Texas waterflood. NACE paper no. 119."

API (1982). "Recommended Practice for Biological Analysis of Subsurface Injection Waters, American Petroleum Institute, Washington, D.C.,."

Arnold, M., & Kajolinna, T. On-line measurement and removal of biogas trace compounds. VTT, Technical Research Centre of Finland.

ASME B31.8-2007, Gas Transmission and Distribution Piping Systems, New York, NY: The American Society of Mechanical Engineers, 2007.

ASTM D2513, Standard Specification for Thermoplastic Gas Pressure Pipe, Tubing and Fittings, West Conshhohocken, PA: ASTM.

ASTM D3350, Standard Specification for Polyethylene Plastics Pipe and Fittings MaterialsPPI , West Conshhohocken, PA: ASTM.

Bak, F. and P. N. (1991). "Sulfate-reducing bacteria in littoral sediment of Lake Constance." FEMS Microbiol. Ecol. 85: 43–52.

Batista, J. F., R. F. Pereira, J. M. Lopes, M. F. Carvalho, M. J. Feio, M. A. Reis, (2000). "In situ corrosion control in industrial water systems." Biodegradation 11(6): 441-8.

Blackburn, F. E. "Non-Bioassay Techniques for Monitoring SRB." CORROSION/2004 (Houston, TX: NACE, 2004).

Boivin, J., et al. (1990). "The Influence of Enzyme Systems on MIC." CORROSION/90, paper no. 128 (Houston, TX: NACE, 1990).

Bradey, G. S., et al., Materials Handbook, New York: McGraw-Hill Co., 1997, pp. 303-311.

Braker, G., et al. (1998). "Development of PCR Primer Systems for Amplification of Nitrite Reductase Genes (nirK and nirS) To Detect Denitrifying Bacteria in Environmental Samples." Appl. Environ. Microbiol. 64(10): 3769-3775.

Brandrupp, J. and Immergut, E. H., Polymer Handbook, 3rd ed., New York: John Wiley & Sons, 1989, pp. v/7-v/13.

Brinkman, N. E., et al. (2003). "Evaluation of a Rapid, Quantitative Real-Time PCR Method for Enumeration of Pathogenic Candida Cells in Water." Appl. Environ. Microbiol. 69(3): 1775-1782.

Broda, D. M., et al. (2000). "Clostridium algidixylanolyticum sp. nov., a psychrotolerant, xylan- degrading, spore-forming bacterium." Int. J. Syst. Evol. Microbiol. 50 Pt 2: 623-31.

Campaignolle, X. and J.-L. Crolet (1997). "Method for Studying Stabilization of Localized Corrosion on Carbon Steel by Sulfate-Reducing Bacteria." Corrosion 53(6).

Campaignolle, X., et al. (1993). "Stabilization of Localized Corrosion of Carbon Steel by Sulfate-Reducing Bacteria." Nace International Corrosion 93, Paper no. 302: 1-7.

Caumette, P. (1993). "Ecology and physiology of phototrophic bacteria and sulphate-reducing bacteria in marine salterns." Experientia 49: 473-481.

Chan, K. Y., et al. (2002). "Anaerobic electrochemical corrosion of mild steel in the presence of extracellular polymeric substances produced by a culture enriched in sulfate-reducing bacteria." Environ Sci Technol. 36(8): 1720-1727.

Chao, S., & Attari, A. (1995). Characterization and Measurement of Natural Gas Trace Constituents. Des Plaines: Institute of Gas Technology.

Chao, S., Crippen, K., & Janos, A. (1999). Analysis of Trace Level Compounds in Natural Gas. Des Plaines: Institute of Gas Technology.

Crippen, K., Janos, A., Bora, R., & Deddo, M. (2003). Sampling and analysis of trace constituents in Canadian Pipeline Gas. Des Plaines: Gas Technology Institute.

Daumas, S., et al. (1993). "Measurement of the net production of acidity by a sulphate-reducing bacterium: experimental checking of theoretical models of microbially influenced corrosion." Res. Microbiol. 144(4): 327-332.

de Franca, F. P. and M. T. S. Lutterbach (1996). "Variation in sessile microflora during biofilm formation on AISI-304 stainless steel coupons." Journal of industrial microbiology 17(1): 6-10.

Dewil, R., Appels, L., & Baeyens, J. (2006). Energy use of biogas hampered by the presence of siloxanes. Energy Conversion and Management , 1711–1722.

Dick, John S., ed., Rubber Technology Compouding and Testing for Performance. Cincinnati: HanserGardner Publications, 2001, pp. 1-69.

Dickinson, W. H. and Z. Lewandowski (1996). "Manganese Biofouling and the Corrosion Behavior of Stainless Steel." Biofouling 10: 79-93.

Dorsey, M. H., et al. (2002). "Monitoring for Corrosion and Microbiological Activity in a Cooling Water System." Plant Power Chemistry 4(12): 721-731.

Dubey, R. S. and Upadhyay (2001). "Microbial Corrosion Monitoring by an Amperometric Microbial Biosensor Developed using Whole Cell of Pseudomonas sp." Biosensors and Bioelectronics 16: 995-1000.

Dzierzewicz, Z., B. Cwalina, L. Weglarz, S. Glab (1992). "Isolation and evaluation of corrosive aggressivity of wild strains of sulfate reducing bacteria." Acta Microbiologica Polonica 41: 211-221.

Dzierzewicz, Z., et al. (1997). "The relationship between microbial metabolic activity and biocorrosion of carbon steel." Res. Microbiol. 148(9): 785-93.

Eashwar, M. and S. Maruthamuthu (1995). "Mechanism of biologically produced ennoblement: Ecological perspectives and a hypothetical model." Biofouling 8(3): 203-213.

Emde, K. M. E., D. W. Smith, and R. Facey (1992). "Initial investigation of microbially influenced corrosion (MIC) in a low temperature water distribution system." Water Research 26: 169-175.

Emerson, D. and W. C. Ghiorse (1992). "Isolation, Cultural Maintenance, and Taxonomy of a Sheath-Forming Strain of Leptothrix discophora and Characterization of Manganese-Oxidizing Activity Associated with the Sheath." Appl. Environ. Microbiol. 58: 4001-4010.

Farthing, S. (1997). "Company combats MIC with aggressive control program." Pipeline and Gas Industry Oct '97: 43-47.

Geesey, G. G. (1991). Biofouling and biocorrosion in industrial water systems. H.-C. Flemming and G. G. Geesey. Berlin, Springer-Verlag.

Gent, Alan N., ed. Engineering with Rubber, How to Design Rubber Components, Cincinnati: HanserGardner Publications, 2001.

Gibson, G. R. and X. Wang (1994). "Regulatory effects of bifidobacteria on the growth of other colonic bacteria." J. Appl. Bacteriol. 77(4): 412-20.

Gonzalez, J. L., et al. (1997). "A damage model for assessing pipeline safety in corrosion environments." Corrosion 53: 935-943.

Graves, J. W., E. H. Sullivan (1996). "Internal corrosion in gas gathering systems and transmission lines." Materials Protection 5: 33-37.

GRI Report: 99/0039, Technical Reference on the Physical, Mechanical, and Chemial Properties of PA11 Pipe Materials for Use in Gas Distribution Systems Operating at High Pressures and Temperatures, Des Plains, IL: Gas Technology Institute, 1999.

Gu, T., et al. (2009). "A New Mechanistic Model for MIC Based on a Biocatalytic Cathodic Sulfate Reduction Theory, NACE/09, Paper 09390."

Guy, R. A., et al. (2003). "Real-Time PCR for Quantification of Giardia and Cryptosporidium in Environmental Water Samples and Sewage." Appl. Environ. Microbiol. 69(9): 5178-5185.

Hamilton, W. A. (1985). "Sulphate-reducing bacteria and anaerobic corrosion." Ann. Rev. Microbiol. 39: 195-217.

Hamilton, W. A. (1985). "Sulphate-reducing bacteria and anaerobic corrosion." Annu. Rev. Microbiol. 39: 195-217.

Hanert, H. H. (1981). The genus Siderocapsa (and other iron- or manganese-oxidizing eubacteria. The Prokaryotes, a Handbook on Habitats, Isolation and Identification of Bacteria. M. Starr, H. Truper, A. Balows and Schlegel. New York, Springer-Verlag. 3: 1049-1059.

Hardy, J. A. and J. L. Brown (1984). "The Corrosion of Mild Steel By Biogenic Sulfide Films Exposed to Air." Corrosion 40(12): 650.

Harmsen, H. J., et al. (1996). "Population dynamics of propionate-oxidizing bacteria under methanogenic and sulfidogenic conditions in anaerobic granular sludge." Appl. Environ. Microbiol. 62(6): 2163-8.

Heitz, E. (1996). Microbially influenced corrosion of materials. E. Heitz, H.-C. Flemming and W. Sand. Berlin, New York, Springer-Verlag.

Hemmingsen, T., et al. (1992). "Formation of Ferrous Sulfide Film from Sulfite on Steel Under Anaerobic Conditions." Corrosion 48(6): 475-481.

Hernandez, G., et al. (1994). "Corrosion Inhibition of Steel by Bacteria." Corrosion 50(08): 603-608.

Hernández-Gayosso, M. J., et al. (2004). "Microbial consortium influence upon steel corrosion rate, using the electrochemical impedance spectroscopy technique." Materials and Corrosion 55(9): 676-683.

Hines, M. E., et al. (2002). Sulfur cycling. Manual of Environmental Microbiology. L. D. Stetzenbach. Washington, DC, ASM Press: 427-438.

Horn, J. and D. Jones (2002). Microbiologically influenced corrosion: Perspectives and approaches. Manual of environmental microbiology. C. J. Hurst, Crawford, R. L., Knudsen, G. R., McInerney, M. J., Stetzenbach, L. D. Washinton, DC, ASM Press: 1072-1083.

http://en.wikipedia.org/wiki/Bacillus_licheniformis.

Ibekwe, A. M., et al. (2002). "Multiplex Fluorogenic Real-Time PCR for Detection and Quantification of Escherichia coli O157:H7 in Dairy Wastewater Wetlands." Appl. Environ. Microbiol. 68(10): 4853-4862.

Jack, T. R. (2001). MIC in Underground Environments: External Corrosion in the Gas pipeline Industry. A Practical Manual on Microbiologically Influenced Corrosion. J. G. S. II. Houston, TX, NACE International. 2: 6.1-6.42.

Jack, T. R. and M. J. Wilmott (1995). "Indicator Minerals Formed During External Corrosion of Line Pipe." MP 34: 19.

Jack, T. R., et al. (1994). Microbiologically Influenced Corrosion Testing. The Characterization of Sulfate-Reducing Bacteria in Heavy Oil Waterflood Operations. J. R. Kearns and B. J. Little, Philadelphia, PA: ASTM: 108.

Jack, T. R., et al. (1998). "Corrosion Consequences of Secondary Oxidation of Microbial Corrosion." Corrosion 54(3): 246-252.

Jayaraman, A., et al. (1997). "Axenic Aerobic Biofilms Inhibit Corrosion of SAE 1018 Steel through Oxygen Depletion." Applied Microbiology and Biotechnology 48: 11-17.

Jayaraman, A., et al. (1999). "Axenic aerobic biofilms inhibit corrosion of copper and aluminum." Appl Microbiol Biotechnol. 52(6): 787-790.

Jayaraman, A., et al. (1999). "Inhibiting sulfate-reducing bacteria in biofilms on steel with antimicrobial peptides generated in situ." Appl Microbiol Biotechnol. 52(2): 267-275.

Johnson, Peter S., Rubber Processing an Introduction, Cincinnati: HanserGardner Publications, 2001, pp. 137-140.

Kajdasz, R. E., et al. (1984). "Biocidal Efficacy with Respect to Sessile and Planktonic." IWV: 85-93.

Kanauchi, O., et al. (1999). "Increased growth of Bifidobacterium and Eubacterium by germinated barley foodstuff, accompanied by enhanced butyrate production in healthy volunteers." Int. J. Mol. Med. 3(2): 175-9.

Kane, R. D. and S. Campbell (2004). "Real-Time Corrosion Monitoring of Steel Influenced by Microbial Activity (SRB) in Simulated Seawater Injection Environments." CORROSION NACExpo'04: NACE Paper 04579.

Kasahara, K. and F. Kajiyama (1986). "Role of Sulfate Reducing Bacteria in the Localized Corrosion of Buried Pipes." Biologically Induced Corrosion; Proceedings of the International Conference on Biologically Induced Corrosion. June 10-12, 1985, Gaithersburg, Maryland. Houston, TX, NACE.: 171-183.

Kholodenko, V. P., S. K. Jigletsova, V. A. Chugnov, V. B. Rodin, V. S. Kobelev, S. V. Karpov (2000). "Chemicomicrobiological diagnostics of stress corrosion cracking of trunk pipelines." Appl. Biochem. Microbiol. 36: 594-601.

King, L. D. and J. D. A. Miller (1971). "Corrosion by the sulfate reducing bacteria." Nature 233: 491-492.

King, R. A. and D. S. Wakerley (1973). "Corrosion of Mild Steel by Ferrous Sulphide." Br Corros J 8(1): 41-45.

King, R. A., et al. (1973). "Corrosion of mild steel by iron sulfides." Brit Corrosion J 8: 137-142.

King, R. A., et al. (1973). "Corrosion of Mild Steel in Cultures of Sulphate-Reducing Bacteria: Effect of changing the soluble iron concentration during growth." Br Corros J 8(2): 89-93.

Le Borgne, S., et al. (2007). "Detecting and Monitoring Bacteria in Seawater Injection Systems." Materials Performance 46(11): 52-56.

Li, S. Y., et al. (2001). "Microbiologically Influenced Corrosion of Carbon Steel Exposed to Anaerobic Soil." Corrosion 57(9): 815-828.

Little, B. and R. Ray (2002). "A Perspective on Corrosion Inhibition by Biofilms." Corrosion 58(5): 424-428.

Little, B. J. and P. Wagner (2001). Microbiologically Influenced Corrosion. Peabody's Control of Pipeline Corrosion. R. L. Bianchetti, Houston, TX: NACE, 2001.

Little, B. J., et al. (1998). "The role of biomineralization in microbiologically influenced corrosion, p. 294/1–294/18. In Proceedings of the CORROSION/98 Research Topical Symposia. NACE International, Houston, TX."

Little, B. J., et al. (2000). "Relationship Between Corrosion and the Biological Sulfur Cycle: A Review." Corrosion 56(4): 433.

Little, B. J., et al. (2006). "Diagnosing Microbiologically Influenced Corrosion: A State-of-the-Art Review." Corrosion(62): 1006.

Little, B., et al. (1991). "Microbiologically Influenced Corrosion of Metals and Alloys." Int. Mat. Rev. 36(6): 253-272.

Little, B., et al. (1991). "Microbiologically Influenced Corrosion of Metals and Alloys." Int. Mat. Rev. 36(6): 253-272.

Lockwood, S. F., et al. (1993). "Microbiologically lnfluenced Corrosion in the Natural Gas Industry." Topical Report (Unpublished) to Gas Research Institute, February 1993.

Lutey, R. W. (1993). Treatment for the Mitigation of MIC. A Practical Manual on Microbiologically Influenced Corrosion. J. G. Stoecker, Houston, TX: NACE: 9.1-9.9.30.

Maidak, B. L., et al. (2001). "The RDP-II (Ribosomal Database Project)." Nucleic Acids Res 29(1): 173-4.

Mantell, C. L., ed., Engineering Materials Handbook, New York: McGraw-Hill Co., 1958, pp. 32-132.

Mason, Jim, et al., Technical Review of Rilsan® Polyamide-11 High Pressure Natural Gas Pipe Field Installations, Coiling Studies and Pipe Durability Testing, Plastics Pipes XII, Milan, Italy, April. 19-22, 2004.

McCafferty, E. and J. V. McArdle (1992). Corrosion Inhibition by Biological Siderophore. 182nd Society Meeting (Toronto, Canada: The Electrochemical Society).

McCoy, W. F. (1987). Strategies for the treatment of biological biofouling. Biological fouling of industrial water systems - A problem solving approach. M. W. Mittelman and G. G. Geysey. San Diego, CA, Water Micro. Associates: 247-268.

McKetta, John J. ed., Encyclopedia of Chemical Processing and Design, New York: 1985, pp. 369-372.

McNeil, M. B. and B. J. Little (1990). "Mackinawite Formation During Microbial Corrosion,." Corrosion 46: 599-600.

Mudryk, Z. J., et al. (2000). "The occurrence and activity of sulphate-reducing bacteria in the bottom sediments of the Gulf of Gdañsk." OCEANOLOGIA 42(1): 105-117.

Muyzer, G., et al. (1993). "Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA." Appl Environ Microbiol 59(3): 695-700.

Myers, C. and K. H. Nealson (1988). "Bacterial Manganese Reduction and Growth with Manganese Oxide as the Sole Electron Acceptor." Science 240: 1319.

NACE "Field Monitoring of Bacterial Growth in Oilfield and Gas Systems." NACE Standard TM0194-2004.

NACE (2006). "Detection, Testing, and Evaluation of Microbiologically Influenced Corrosion (MIC) on External Surfaces of Buried Pipelines." NACE Standard TM0106-2006.

Nemati, M., et al. (2001). "Impact of nitrate-mediated microbial control of souring in oil reservoirs on the extent of corrosion." Biotechnol. Prog. 17(5): 852-9.

Nichols, W. W., et al. (1989). "The penetration of antibiotics into aggregates of mucoid and non-mucoid Pseudomonas aeruginose." J. Gen. Microbiol. 35(35): 1219-1303.

Nordstrom, D. K. and G. Southam (1997). Geomicrobiology of Sulfide Mineral Oxidation. Reviews in Mineralogy Geomicrobiology: Interactions Between Microbes and Minerals. J. F. Banfield and K. H. Nealson, Washington, DC: Mineralogical Society of America. 35: 361-390.

Ohm, Robert F., ed, The Vanderfilt Rubber Handbook, Norwalk: R. T. Vanderbilt Company Inc., 1990.

Osburne, M. S., T. H. Griossman, P. R. August, I. A. MacNeil (2000). "Tapping into microbial diversity for natural products drug discovery." ASM News 66: 411-417.

Osswald, T. A., and Menges, G., Materials Science of Polymers for Engineers, Cincinnati: HanserGardner Publications, 2003.

Pawlowska, M., Czerwinski, J., & Stepniewski, W. (2008). Variability of the Non-Methane Volatile Organic Compounds (NMVOC) Composition in Biogas from Sorted and Unsorted Landfill Material. Archives of Environmental Protection , 34 (3), 287-298.

PDL, Handbook of Plastic Joining (A Practical Guide), Norwich, NY: Plastics Design Library (PDL), 1997.

Peacock, Andrew J., Handbook of Polyethylene: Structures, Properties and Applications, New York: Marcel Dekker, Inc, 2000.

Pope, D. H. (1992). "State-of-the-Art Report on Monitoring, Prevention and Mitigation of Microbiolwically lnfluenced Corrosion in the Natural Gas Industrv." Topical Report, GRI-9210382. Gas Research Institute, Chicago, IL.

Pope, D. H. (2001). Microbiologically Influenced Corrosion of Internal Aspects of Natural Gas Industry Pipelines and Associated Equipment: Mechniasms, Diagnosis, and Mitigation. A Practical Manual on Microbiologically Influenced Corrosion. J. G. S. II. Houston, TX, NACE International. 2: 6.13-6.25.

Pope, D. H. and E. A. M. III (1995). "Some Experiences With Microbiologically Influenced Corrosion of Pipelines." MP 34(5): 23.

Pope, D. H. and R. M. Pope (1998). Guide for the Monitoring and Treatment of Microbiologically Influenced Corrosion in the Natural Gas Industry, Gas Research Institute.

Pope, D. H. and R. M. Pope (2001). Microbiologically Influenced Corrosion in Fire Protection Sprinkler Systems. A Practical Manual on Microbiologically Influenced Corrosion. J. G. S. II. Houston, TX, NACE International. 2: 5.1-5.7.

Pope, D. H. and R. Skultety (1995). "Microbiologically lnfluenced Corrosion in Natural Gas Storage Fields: Diagnosis, Monitoring and Control." NACE International Conference on Microbiologically lnfluenced Corrosion, Paper No. 57. New Orleans, LA.

Pope, D. H., et al. (1988). "Organic Acid Corrosion of Carbon Steel: A Mechanism of Microbiologically Influenced Corrosion." In Proceedings of the NACE International Annual Conference, Corrosion/1988, Paper #79. Houston, TX.

Pope, D. H., et al. (1989). "Mitigation strategies for microbially influenced corrosion in gas industry facilities." Corrosion '89: NACE paper 192.

Pope, D. H., R. M. Pope (1998). Guide for the monitoring and treatment of microbiologically influenced corrosion in the natural gas industry., Gas Research Institute.

Pope, D. H., T. P. Zintel, B. A. Cookingham, R. G. Morris, D. Howard, R. A. Day, J. R. Frank, and G. E. Pogemiller (1989). "Mitigation strategies for microbially influenced corrosion in gas industry facilities." Corrosion '89: NACE paper 192.

Pots, B. F. M. and S. D. Kapusta (2005). "Prediction of Corrosion Rates of the Main Corrosion Mechanisms in Upstream Applications." Nace International Corrosion 2005. Paper No. 05550.: 1-21.

PPI Report: TN-15/2009, Resistance of Solid Wall Polyethylene Pipe to a Sanitary Sewage environment, Washington DC: Plastic Pipe Institue (PPI), 2009.

PPI Report: TN-17/2008, Crosslinked Polyethylene (PEX) Pipe & Tubing, Washington DC: Plastic Pipe Institue (PPI), 2008.

PPI, Handbook of Polyethylene Pipe, Washington DC: Plastic Pipe Institue (PPI), 2006, pp. 5-103.

Pridal, A. (2001). "Microorganisms in Dead Bumble Bee Larvae (Bombus spp.) from Laboratory-Reared Colonies." ACTA Universitatis Agriculturae et Silviculturae Mendelianae Brunensis(5): 41-48.

Rasi, S. (2009). Biogas Composition and Upgrading to Biomethane. University of Jyvaskyla.

Rodgers, Brendan, ed., Rubber Compounding Chemistry and Applications, New York: Marcel Dekker, Inc, 2001.

Rosen, S. L., Fundamental Principles of Polymeric Mateirals, New York: John Wiley & Sons, 1982.

Royer, R. A. and R. F. Unz (2002). "Use of Electrical Resistance Probes for Studying Microbiologically Influenced Corrosion." Corrosion 58(10): 863-870.

Saber, D. L., Takach, S. F., & Cruz, K. M. (2009). Pipeline Quality Biomethane: North American Guidance Document for Introduction of Dairy Waste Derived Biomethane into Existing Natural Gas Networks .

Scheldeman, P., et al. (2005). "Incidence and Diversity of Potentially Highly Heat-Resistant Spores Isolated at Dairy Farms 10.1128/AEM.71.3.1480-1494.2005." Appl. Environ. Microbiol. 71(3): 1480-1494.

Scholten, Frans, et al., Polyamide 11, 12 and 6.12 for High-Pressure Gas Pipelines, IGRC 2008, Paris, France, Oct. 8-10, 2008.

Schweitzer, P.A., Corrosion-Resistant Linings and Coatings, New York: Marcel Dekker, Inc, 2001.

Schweitzer, P.A., Mechanical and Corrosion-Resistant Properties of Plastics and Elastomers, New York: Marcel Dekker, Inc, 2000.

Scott, P. J. B. (2004). "Expert Consensus on MIC: Prevention and Monitoring." Materials Performance(3): 2-6.

Shi, X., et al. (2002). "Microbially deposited manganese and iron oxides on passive metals—their chemistry and consequences for material performance." Corrosion 58: 728.

Skovhus, T. L., et al. (2004). "Real-Time Quantitative PCR for Assessment of Abundance of Pseudoalteromonas Species in Marine Samples." Appl. Environ. Microbiol. 70(4): 2373-2382.

Song, F. M. (2008). "Exploring to Predict Pipeline High-pH SCC Crack Growth Rate, Sept. 29-Oct. 3, 2008, ASME IPC 2008, Paper 64671 (A Best Conference Paper)."

Song, F. M. (2008). "Overall Mechanisms of High pH and Near-Neutral pH SCC, Models for Forecasting SCC Susceptible Locations, and Simple Algorithms for Predicting High pH SCC Crack Growth Rates. March 16-20, 2008 (Houston, TX: NACE, 2008) Paper: 08129."

Song, F. M. and N. Sridhar (2006). "A Two-Dimensional Model for Steel Corrosion under a Disbonded Coating due to Oxygen with or without Cathodic Protection-Part 1: Full Numerical Solution." Corrosion 62(8): 676-686.

Song, F. M. and N. Sridhar (2008). "Modeling Pipeline Corrosion under a Disbonded Coating under the Influence of Underneath Flow." Corrosion 64(1): 40-50.

Song, F. M. and N. Sridhar (2008). "Modeling Pipeline Crevice Corrosion under a Disbonded Coating with or without Cathodic Protection under Transient and Steady-State Conditions." Corrosion Science 50(1): 70-83.

Song, F. M., et al. (2002). "CO2 Corrosion of Bare Steel under an Aqueous Boundary Layer with Oxygen." Journal of The Electrochemical Society 149(11): B479-B486.

Song, F. M., et al. (2004). "CO2 Corrosion of Bare Steel under an Aqueous Boundary Layer." Corrosion 60(8): 736-748.

Song, F. M., et al. (2005). "Corrosion and Current Flow within a Pipeline Disc Crevice." Corrosion 62(2): 145-154.

Song, M., et al. (2004). "CO2 Corrosion of Bare Steel under an Aqueous Boundary Layer with Oxygen and Cathodic Protection." Corrosion 60(9): 845-851.

Strickland, L. N., R. T. Fortnum, B. W. DuBose (1996). "A case history of microbiologically influenced corrosion in the Lost Hills oilfield, Kern county California." Corrosion '96: NACE paper 297.

Stults, J. R., O. Snoeyenbos-West, B. Methe, D. R. Lovley, D. P. Chandler (2001). "Application of the 5' fluorogenic exonuclease assay (TaqMan) for quantitative ribosomal DNA and rRNA analysis in sediments." Appl. Environ. Microbiol. 67(6): 2781-2789.

Suzuki, M. T., et al. (2000). "Quantitative Analysis of Small-Subunit rRNA Genes in Mixed Microbial Populations via 5'-Nuclease Assays." Appl. Environ. Microbiol. 66(11): 4605-4614.

Synthetic Rubber: The Story of an Industry, Houston: International Institute of Sythetic Rubber Producers, Inc., pp. 15-20.

Tanji, Y., et al. (2002). "Chemical Analysis of an Artificial Biofilm that Enhances or Inhibits Carbon Steel Corrosion." Corrosion 58(3): 232-239.

Telang, A. J., S. Ebert, J. M. Foght, D. W. S. Westlake, G. E. Jenneman, D. Gevertz, G. Voordouw (1997). "Effect of nitrate injection on the microbial community in an oil field as monitored by reverse sample genome probing." Appl. Environ. Microbiol. 63(5): 1785-1793.

Torsvik, V., et al. (1990). "High diversity in DNA of soil bacteria." Appl Environ Microbiol 56(3): 782-7.

Towers, R. (2000). "Accelerated Corrosion in Cargo Tanks of Large, Double-Hull Ships, Causes and Countermeasures." Protective Coatings Europe(3): 30-42.

Tributsch, H., et al. (1998). "Role of Transient Iron Sulfide Films in Microbial Corrosion of Steel." Corrosion 54(3): 216-227.

Vetting, M. W., et al. (2000). "Structure of Acinetobacter strain ADP1 protocatechuate 3, 4-dioxygenase at 2.2 A resolution: implications for the mechanism of an intradiol dioxygenase." Biochemistry 39(27): 7943-55.

von Wolzogen Kuhr, C. A. H. and L. S. van der Vlugt (1934). "The graphitization of cast iron as an electrochemical process in anaerobic soils." Water Research 18: 147-165.

Voordouw, G. (1998). "Reverse Sample Genome Probing of Microbial Community Dynamics." ASM News 64: 627-633.

Voordouw, G., et al. (1994). Application of Reverse Sample Genoma Probing to the Identification of Sulfate-Reducing Bacteria. Microbiologically Influenced Corrosion Testing, ASTM STP 1232. J. R. Kearns and B. J. Little, Philadelphia, PA: ASTM: 188-199.

Waters, M. S., et al. (2009). "Simultaneous interferometric measurement of corrosive or demineralizing bacteria and their mineral interfaces." Appl Environ Microbiol. 75(5): 1445-1449.

Webster, B. J. and R. C. Newman (1994). ASTM STP 1232: Producing Rapid Sulfate-Reducing Bacteria-Influenced Corrosion in the Laboratory. Microbiologically Influenced Corrosion Testing. J. Kearns and B. J. Little. West Conshohocken, PA, ASTM: 28-41.

White, James L., ed., Rubber Processing, Technology-Materials-Principles, Cincinnati: HanserGardner Publications, 1995, pp. 1-57.

Williams, W. A., et al. (1997). "A phylogenetic analysis of aerobic polychlorinated biphenyl-degrading bacteria." Int. J. Syst. Bacteriol. 47(1): 207-10.

Willoughby, David A., Plastic Piping Handbook, New York, NY: The McGraw-Hill Companies, Inc., 2002, pp. 1.1-2.16

Zellner, G., et al. (1996). "Anaerofilum pentosovorans gen. nov., sp. nov., and Anaerofilum agile sp. nov., two new, strictly anaerobic, mesophilic, acidogenic bacteria from anaerobic bioreactors." Int. J. Syst. Bacteriol. 46(4): 871-5.

Zhu, X. Y. ""Bioreactor/Chemostat System for Rapid Biodemilitarization of Munitions" for DOD/DARPA, subcontracted from SRI. Subcontract No. 41-000810. GTI project No. 20569. Completed in Angust 2008."

Zhu, X. Y. (2007). ""Detection and Mitigation of Microbiologically-Influenced Corrosion (MIC) in Natural Gas Pipelines". GTI Project 20153, completed in March 2007."

Zhu, X. Y. (2007). Rapid Quantification of Butyric Acid-Producing Bacteria Using Real-Time PCR. USA, Gas Technology Institute.

Zhu, X. Y. (2008). Rapid Quantification of Acetic Acid-Producing Bacteria Using Real-Time PCR. USA, Gas Technology Institute.

Zhu, X. Y. and R. D. Joerger (2003). "Composition of microbiota in content and mucus from cecae of broiler chickens as measured by fluorescent in situ hybridization with group-specific, 16S rRNA-targeted oligonucleotide probes." Poult Sci 82(8): 1242-9.

Zhu, X. Y., et al. (2003). "Characterization of microbial communities in gas industry pipelines." Appl Environ Microbiol 69(9): 5354-63.

Zhu, X. Y., et al. (2005). "Application of Quantitative, Real-Time PCR in Monitoring Microbiologically-Influenced Corrosion (MIC) in Gas Pipelines." In Proceedings of the NACE International Annual Conference, Corrosion/2005, Paper #05493. Houston, TX.: 1-20.

Zhu, X. Y., et al. (2006). "Efficacy and risks of nitrate application for the mitigation of SRB-induced corrosion." In Proceedings of the NACE International Annual Conference, Corrosion/2006, Paper #06524. San Diego, CA.: 1-41.

Zhu, X. Y., et al. (2006). "Rapid detection and quantification of microbes related to microbiologically influenced corrosion using quantitative polymerase chain reaction." Corrosion 62: 950-955.

Zhu, X. Y., T. Zhong, Y. Pandya, R. D. Joerger (2002). "16S rRNA-based analysis of microbiota from the cecum of broiler chickens." Appl. Environ. Microbiol. 68(1): 124-37.

Zintel, T. P., et al. (2001). Techniques for MIC Monitoring. A Practical Manual on Microbiologically Influenced Corrosion. J. G. S. II. Houston, TX, NACE International. 2: 10.1-10.19.

Zuo, R. (2007). "Biofilms: strategies for metal corrosion inhibition employing microorganisms." Appl Microbiol Biotechnol. 76(6): 1245.

Zuo, R. and T. K. Wood (2004). "Inhibiting mild steel corrosion from sulfate-reducing and iron-oxidizing bacteria using gramicidin-S-producing biofilms." Appl Microbiol Biotechnol. 65(6): 747-753.

Zuo, R., et al. (2004). "Inhibiting mild steel corrosion from sulfate-reducing bacteria using antimicrobial-producing biofilms in Three-Mile-Island process water." Appl Microbiol Biotechnol. 64(2): 275-283.

End of Report